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CONTRIBUTION TO THE KNOWLEDGE OF COLLOID-CHEMISTRY OF THE GLUTEN

Preliminary Communication

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In the last few years colloid-chemistry has entered into every branch of industry, hence there have been many attempts to use this new science in the flour and baking industry in order to get a closer insight into the greatly complicated phenomena that are manifested in the growth of wheat and in the flour ground from it.

It would be impossible to mention all the research work done by many chemists in various countries of the world. The results gained by them do not correspond and until now the jig-saw puzzle of the baking-power problem is far from being solved. Yet it may be of future value that all substances are extensively tested with regard to their properties, both separately and in combinations. Altho what follows may not be of direct practical use for industry, it will probably give a clearer insight into the colloid-chemical problems of gluten.

On a closer view, the solutions obtained by shaking gluten with acid showed some remarkable phenomena when diluted alkali was added. By the addition of diluted alkali a milky cloudiness appeared, which, superficially seen with the naked eye, reminded us of a flocculation, but microscopically it appeared entirely different.

Before proceeding to a further description of the facts observed, we prefer to explain the method by which these solutions are obtained. For this purpose 3 grams of a very pure and finely powdered gluten preparation (dried in vacuo at a temperature below 30°C.) were shaken in a thermostat (25°C.) with a quantity of water and hydrochloric acid for 45 minutes so that the resulting pH of the protein solution, after centrifugation and filtration, was 4.6. The solution obtained in this

way contained 113.4 milligrams of protein per 10 cc. of solution, determined by the Kjeldahl method. The accuracy of this method of preparation was such that the viscosity of solutions made on different days differed, at most, 2 in the third decimal, measured with an Ostwald viscosimeter at 25°C.

The protein solution obtained in this way showed no microscopical image save for a few defilements. This liquid shows us an entirely different image by adding strongly diluted sodium hydroxide. With the first traces of alkali added, the sol becomes slightly opaque, and after further addition opalescent, then cloudy, after which trails begin to appear in the solution and in the foam. On adding more hydroxide, it becomes opalescent for a second time, and subsequently becomes clear. The microscope gave us a better insight into what happened. Under the greatest magnification the microscopical image of such an opalescent solution—by adding a trace of alkali to the original liquid—gave an impression of innumerable very small particles in the field of vision. These particles showed a vivid Brownian movement.

Further addition of sodium hydroxide made the aspect more distinct, and little globules appeared in the liquid. On adding more, the visible number increased, the drops grew larger in size, the number of particles performing the Brownian movement decreased. Upon increasing the hydroxide concentration the drops grew larger, their own mobility in the surrounding liquid decreased, they began to adhere to the object-glass and fill the field of vision and were distinctly visible under small magnification. Near this state of the process flocculation takes place, that is, protein strings begin to appear in the foam. If the solution is brought quickly past the flocculation point—by now pouring the protein solution into the hydroxide solution—the globules become smaller. By adding more alkali both size and visible number decrease, after which, by a certain concentration of hydroxide, the solution changes again into an optically practically empty liquid. A schedule of our observations, adding alkali to the protein solution, will be found in Table I.

TABLE I

Appearance of the liquid	Clear	Faintly opalescent	Opalescent	Cloudy	Very cloudy		Cloudy	Clear
Microscopical	Optically void	Particles on the verge of being visible	Particles visible 1 μ Brownian movement	Drops clearly visible $\pm$ 2 μ	Drops adhering to the slide Not adhering 4 μ	Protein strings in the foam	Great number of particles $\pm$ 2 μ	Optically void

Increasing quantities of alkali in progressing from left to right.

The phenomena described above manifest themselves in a solution by changing the hydroxyl-ion concentration, on adding sodium hydroxide. When we consider this from the point of view of colloid-chemistry, we see these phenomena as a decrease of hydration in consequence of a decrease of charge until the flocculation point (isoelectric point) is reached and afterwards the particles get an increasing charge, this time negative and coupled with an increased hydration.

Besides this process, which up till now was considered as taking place on the particle itself so that by the decrease of charge the hydration of each particle decreased, there is another factor to be observed in this case. It is evident that the decrease of hydration is not limited to the particle, but before flocculation manifests itself, a joining of particles takes place and these are separated in the form of a new phase.

The following question now arises:

Are these globules of solid or of liquid nature? The perfectly circular form suggests emulsions and therefore liquid nature. That they really are drops of liquid can be confirmed in several ways. The particles, in even the most cloudy solutions, can be filtered through filter paper. As the size of pores of this paper was stated to be 1-2 μ, and drops of 8 μ were measured microscopically before as well as after filtration of the liquid, the globules must necessarily have worked their way through the pores of this paper. A more convincing proof of their power of changing their form and their liquid nature, was found in the fact that the cloudy solutions even filtered through porous crucibles, which have much smaller pores, whereas before and after filtration the cloudiness of the solutions was the same and no substance remained at the filter-bottom of the crucible.

By means of a special method globules were obtained which were distinctly visible under small magnification. By carefully placing a needle point on the covering glass in the vicinity of such a drop, the drop can be deformed, whereas after releasing the gentle pressure, the drop took its original form. At a more vigorous pressure the drop either decomposed into a large number of small drops or flattened to trails on the object glass. Moreover, after leaving a very cloudy solution for several days, a white precipitate settled on the bottom of the test glass. With the naked eye one could see that this sediment consists of a large number of drops. So here is a case of a second liquid phase separating itself out of a liquid, owing to a decrease of charge and hydration of the particles; therefore a case of unmixing a liquid into two liquid phases; an analogy of the system phenol-water,

but with this difference, that in the last mentioned case a true solution can unmix itself, whereas in the case under discussion a colloid solution does the same.

It now remains to be proved that such a system behaves in some of its further properties like the system phenol-water. In order to prove this, let us first consider the composition of the drops. That these drops are relatively rich in protein in comparison with the surrounding liquid is most easily proved by coloring them. This gives us for the present qualitatively a line of action. A following part of our publication will deal quantitatively with this matter. It appears that by carefully coloring a microscopical preparation of a cloudy protein solution with diluted iodine solution, the drops are colored chiefly dark by the iodine, whereas the liquid is colored only slightly yellow. For the time being we can draw the conclusion that the drops contain a considerable quantity of protein, so they can be compared with the phenol-layer in the system phenol-water. There are, moreover, some other properties of such a system, which strike us at once.

Just like the system phenol-water, which possesses for every concentration a temperature in its temperature-concentration diagram above which the system is homogeneous, this system has a temperature for every concentration of added electrolyte, above which it is impossible to effect a separation. On heating such a milky, cloudy solution, the cloudiness disappears and the solution becomes practically clear above a certain temperature, whereas by cooling the warm solution to room temperature, it becomes cloudy again.

Just as by adding a third component, which mixes in every proportion with the two components, to the system phenol-water, the margin of the heterogeneous area is forced down so it is possible to find organic substances that do the same with this system. For instance, a milky, cloudy protein solution becomes clear when acetone is added till the concentration is $30\pm\%$ in the liquid. Alcohol-glycerin and cane-sugar show the same phenomena. However, by adding more acetone to a concentration of $50\pm\%$, the solution becomes cloudy for the second time. The same takes place with higher alcohol-concentration ($80\pm\%$).

This process can easily be followed under the microscope. When adding acetone the drops become less clear, and lose their outlines, and by further increasing the acetone concentration they disappear altogether, till at a concentration of $50\pm\%$ acetone in the solution, the drops reappear. A second time a new liquid phase appears, and the phases are immiscible.

By adding alkali, on the other hand, the change of the hydroxyl-ion concentration was the cause of the appearance of a new liquid phase, so it is possible to separate the original liquid at a constant pH by adding electrolytes. The addition of different electrolytes gave the same phenomena as mentioned above. The separation of the protein sol by adding electrolytes was studied viscosimetrically to ascertain whether the separation of the liquid gave the same results as the discharging and dehydration of other emulsoids.

For the viscosity determination, protein solutions were made by adding a known quantity of electrolyte to 10 cc. of the protein solution, always making up to the same final volume (20 cc.) The viscosities of the solutions were determined in an Ostwald viscosimeter at 25°C. The viscosities are not corrected for the viscosity of the salt solution with a concentration of less than 15 millimoles of electrolyte, hence a correction of the viscosity of the sol need not be brought into account. The retrogression of the viscosity of the solution during a series of measurements was negligible. Table II gives the results of adding different electrolytes to the protein sol.

On recording these values graphically, no deviating results, compared with other known emulsoids, were found. With a small concentration of electrolytes the viscosity falls rapidly. The drop in the viscosity with the same concentration of different electrolytes depends on the valency of the anion. As the kations of the salts are the same, it is not necessary to consider the influence of the latter. It appears, moreover, that the lyotropic influence of the univalent ions manifests itself very strongly and these salts show us a greatly spreading bundle of curves. As the protein is positively charged at this pH, it may be expected that the lyotropic series—SO_4, Cl, Br, NO_3, I, CNS—will manifest itself, in which the SO_4 ion has the weakest power of salting out; the I ion the strongest.

It appears, however, that the SO_4 ion does not behave viscosimetrically as if it belonged to the lyotropic series, but behaves clearly as a bivalent negative ion. It is evident that, on comparing a series of electrolytes for their lyotropic influence, one may consider only those salts that have a similar valency of the active ion. For that reason, one cannot get a correct impression of the true lyotropy with K_2SO_4, as the SO_4 ion finds itself in the above mentioned case, the same holds good for K_4FeCy_6, with which the valency is decisive; and the lyotropic influence can only be determined in comparison with other ions of the same valency. Evidently the effects of the lyotropy of these ions are greatly outbalanced by the passing of the ion to a higher valency.

TABLE II

KCl	10 cc. sol. diluted to 20 cc. η = 1.105			
η	1.090	1.081	1.073	1.068
Millimoles of electrolyte	2.5	5	7.5	10
Appearance	clear	l. op.	op.	cloudy
% decrease in visc.	85.7	77.1	69.5	64.8
KNO_3				
η	1.085	1.074	1.063	1.053
Millimoles of electrolyte	2.5	5	7.5	10
Appearance	clear	l. op.	op.	cloudy
% decrease in visc.	80.9	70.5	60.—	50.5
KI				
η	1.071	1.056	1.040	1.031
Millimoles of electrolyte	2.5	5	7.5	10
Appearance	op.	sl. clo.	clo.	clo.
% decrease in visc.	67.6	53.3	38.1	29.5
$\frac{K_2SO_4}{2}$				
η	1.082	1.059	1.048	1.042
Millimoles of electrolyte	1.5	3	5	7.5
Appearance	clear	op.	sl. clo.	clo.
% decrease in visc.	78.1	56.2	45.7	40
$\frac{K_4FeCy_6}{4}$				
η	1.097	1.071	1.059	1.050
Millimoles of electrolyte	0.1	0.25	0.4	0.5
Appearance	v. l. op.	l. op.	op.	sl. cl.
% decrease in visc.	92.4	67.6	56.2	47.6
NaOH				
η	1.094	1.083	1.075	1.062
Millimoles of electrolyte	0.125	0.25	0.375	0.5
Appearance	clear	clear	sl. op.	op.
% decrease in visc.	89.5	79.1	71.4	59.1

In the next publication we will treat extensively, both with the gluten itself and with the separate components, the influence of alcohol on mixed and unmixed protein solutions and other factors.

THE INFLUENCE OF ENVIRONMENT ON THE MOISTURE CONTENT OF FLOUR AND WHEAT

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Moisture is an essential constituent of wheat and of flour milled from wheat. The moisture of a given wheat may be increased or decreased in the conditioning processes and the moisture content of a flour need not be the same as that of the wheat from which it is milled. But however it is conditioned or milled, flour must contain some moisture and this has a great influence on keeping and baking properties. The first part of the work about to be described deals with the equilibrium existing between the moisture in flour and that in the atmosphere. At first sight this may seem trivial, but when one considers the enormous amount of flour that is prepacked and may be stored in all sorts of atmospheres for several months, the importance of a knowledge of such equilibrium is evident. The earlier part of the work was a direct determination of moisture changes that actually occurred in storage; this led to an investigation of weight changes that took place when certain atmospheric conditions were closely imitated in the laboratory and on which a successful appeal against a conviction for short weight was fought. In Parts III and IV an endeavor has been made to correlate moisture content with relative humidity to find out under what atmospheric conditions the moisture content could be kept constant. In view of the incidence of mites and of fungoidal contamination, which are always to be associated with the storage of damp flour, the findings might be of great value. It is unlikely that mites would develop in flour stored in an atmosphere with a relative humidity of 50% or less, as this would ultimately lead to a moisture content of less than 11%, and such an atmosphere could readily be obtained artificially.

Finally, the question of the interchange of moisture between different wheats on lying together has been investigated.

Part I. Variation in Weight of Packed Flour in Relation to Atmospheric Humidity

Exposure of Bags of Flour in the Laboratory

It has long been known that packed flour can vary in weight. In hot dry weather it loses weight and in wet weather it generally gains in weight. This fact was realized in the trade, and, as the variations were

recognized as being caused by more or less moisture, no one was especially alarmed. With the enforcement in England of the Sale of Food (Weights and Measures) Act, 1926, the position was altered considerably, and the possibility arose of completely erroneous conclusions being drawn from the weight of bags of flour that had been packed for some time and exposed to the varying influences of the atmosphere. Therefore it was considered advisable to investigate, as thoroly as possible, the changes in weight and moisture content of bags of flour over relatively long periods and, if possible, to correlate these changes with the relative humidity of the atmosphere. Experiments started on August 11, 1927, were carried out for 14 months, during which the atmospheric conditions varied considerably. On one occasion the atmosphere was absolutely saturated for several days and later there was a period of low humidity and high temperature for about 18 days.

Procedure

Bags of flour were weighed to within 0.1 gm. and exposed in the laboratory on a shelf on which was fixed a hygrometer. In the first, second, and fourth series 12 bags were exposed; each week one bag was opened and the moisture determined. In this way a record was obtained of the average weight of the bags each week, the determined moisture content, and the relative humidity of the atmosphere. In the third series the same procedure was adopted with 28 bags instead of 12.

The results obtained have not been tabulated but are shown graphically in Figures 1, 2, 3, and 4.

Discussion

First Series

In the series there were two distinct weather periods. In the first period (August 11-September 29) even fluctuations of humidity occurred and there was little change in the weight of the bags. The total variation was less than 1%. On September 29 the humidity of the atmosphere rose sharply from 50% to 100% and remained at that point for two days and above the average for 9 days. The weight of the bags increased by 10 gm. (on an original of 459 gm.) i.e., 2.2%.

Second Series

The fluctuations of humidity in this period, January-March, 1928, were very even and after 3 months there was no difference in the weight of the bags, altho there had been a variation of about 1% during the period.

Third Series

In this series a period of exceptional heat and drought was experienced. From April 12 to May 24 humidity fluctuations were fairly regular and the weights were practically constant between 458 and 454

gm. The hot period began on May 24 and the fall in humidity was followed by a loss of weight, until on July 19 a loss of 18.4 gm. or 4.0%, had been recorded. The humidity then rose again and on August 2 the weight had increased by 16.5 gm., so the weight lost in the dry period had practically been recovered.

Fourth Series (3-lb. Cotton Bags)

This was started on June 14 when the relative humidity was low and the temperature high and an immediate loss occurred so that by July 19 the weight had fallen by 3.4%. The determined moisture of the flour fell from 14.25 to 11.3%. Later the weight increased as the humidity rose.

In this series it was found that the variations in weight are about the same in cotton as in paper bags, tho the general belief in the trade is that flour loses more in cotton than in paper bags.

The fluctuations in weight, relative humidity, and moisture content can be readily followed from the graphs (Figs. 1, 2, 3, and 4).

Part II. Experiments in Which Flour Was Subjected to an Atmosphere with Controlled Relative Humidities

The administration of the Sale of Food (Weights and Measures) Act of England led to considerable confusion in the case of prepacked flour. Prosecutions were instituted and convictions made on account of bags of flour being short of the weight declared on the package. The results of the experiments described in Part I led the author to the conclusion that flour containing about 15% moisture could lose 4% or more when exposed in sealed bags to the atmospheric conditions that prevailed in England in June and July, 1928. On July 28 a grocer of Luton (Bedfordshire) was fined for selling bags of flour that showed varying shortages in weight from 1.78% to 4.01% and an average loss of 2.38%. As the flour had been packed and stored during the hot dry weather and was seized by the inspector at the period of lowest humidity of the year, it seemed evident that the whole loss could be accounted for by unavoidable evaporation and the case was considered suitable for appeal. At the request of the defence the author carried out an experiment in an endeavor to prove this. The experiment consisted of exposing bags of the defendant's flour to relative humidity conditions exactly similar to those which prevailed in the Luton district during the period in question. The readings were obtained from the government agricultural station at Rothamsted, about six miles from Luton.

The apparatus consisted of a chamber 3x2x2 feet made of heavily enameled sheet iron and suitably lagged. A small fan was fixed at the back of the chamber and the bearing was made airtight by an oil seal. This fan was merely to circulate the air inside the chamber so as to obtain equilibrium conditions as quickly as possible. The chamber was fitted with a perforated metal shelf on which the bags were placed. The

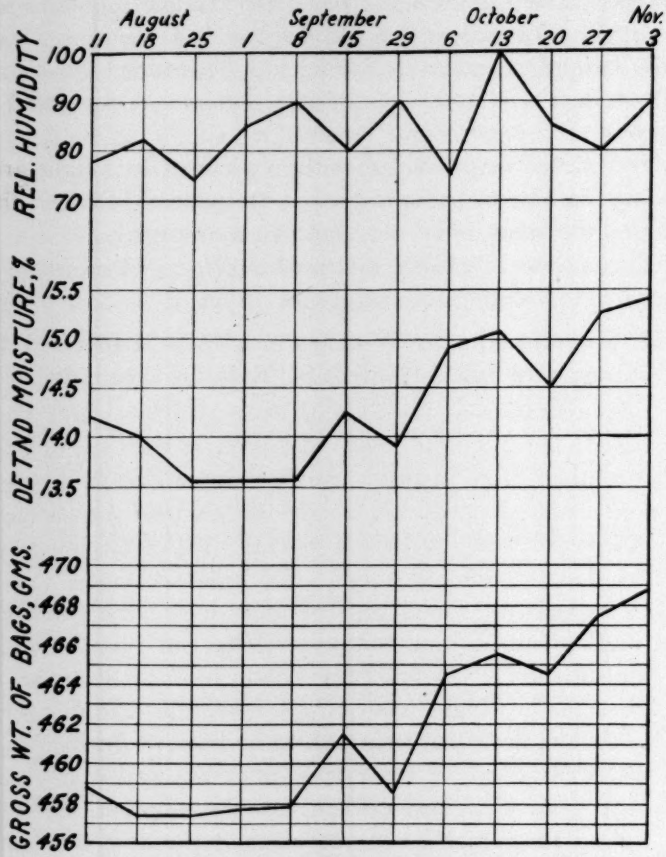

Fig. 1. Series 1. Exposure of Paper Bags, August 11 to November 3, 1927

temperature was controlled by a series of lamps of different voltages. The relative humidity was controlled by the introduction of basins containing sulphuric acid of varying vapor pressure. Preliminary experiments were carried out to obtain the strengths of acid required to give certain relative humidities at 25°C. The method adopted was to introduce sulphuric acid of known dilutions and then to allow the atmosphere to come to equilibrium. When the recording hygrometer was found to be tracing a straight horizontal line the acid was taken out and titrated. The results obtained are shown in Table I.

TABLE I

APPROXIMATE STRENGTH OF H_2SO_4 REQUIRED TO GIVE DIFFERENT HUMIDITIES

Relative humidity	Strength of acid
%	%
10	65.2
15	61.0
20	59.5
25	56.5
30	54.2
35	51.5
40	48.5
45	45.0
50	43.0
55	40.0
60	38.0
65	36.0
70	33.5
75	31.0
80	27.0
85	24.0
90	15.0
95	10.0

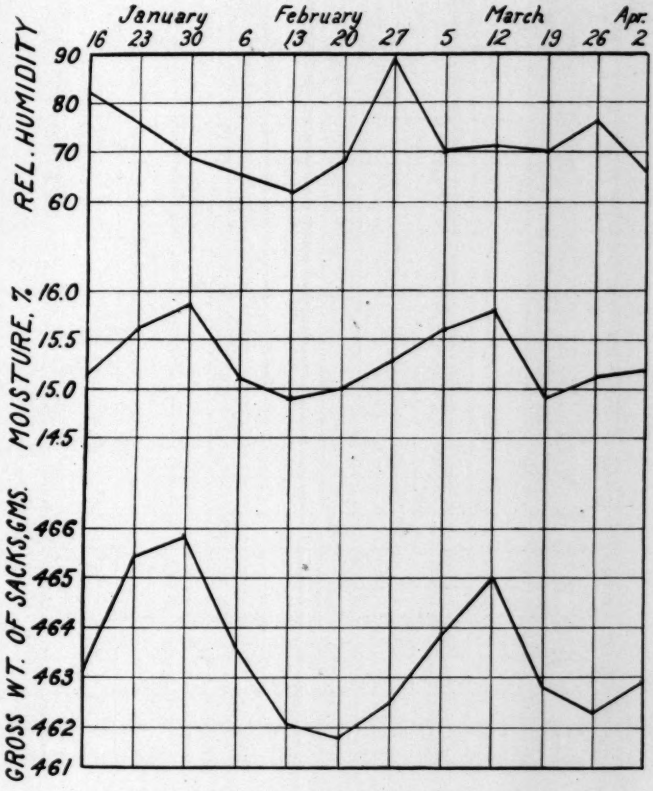

Fig. 2. Series 2. Exposure of One-Pound Paper Bags

It is not claimed that these figures are absolutely accurate, as the temperatures varied during the trials, and for an absolute value it would be necessary to have more adequate lagging of the chamber and more accurate thermostatic control. This was not possible in the time available owing to the urgency of the case. Moreover, the figures were obtained only as a guide, as it was realized that the moisture content of the flour to be introduced would have some influence on the relative humidity of the chamber.

Having obtained these approximate data the test was begun on September 17, 1928. The results obtained are shown graphically in Figure 5. The full line in the relative humidity curve represents the

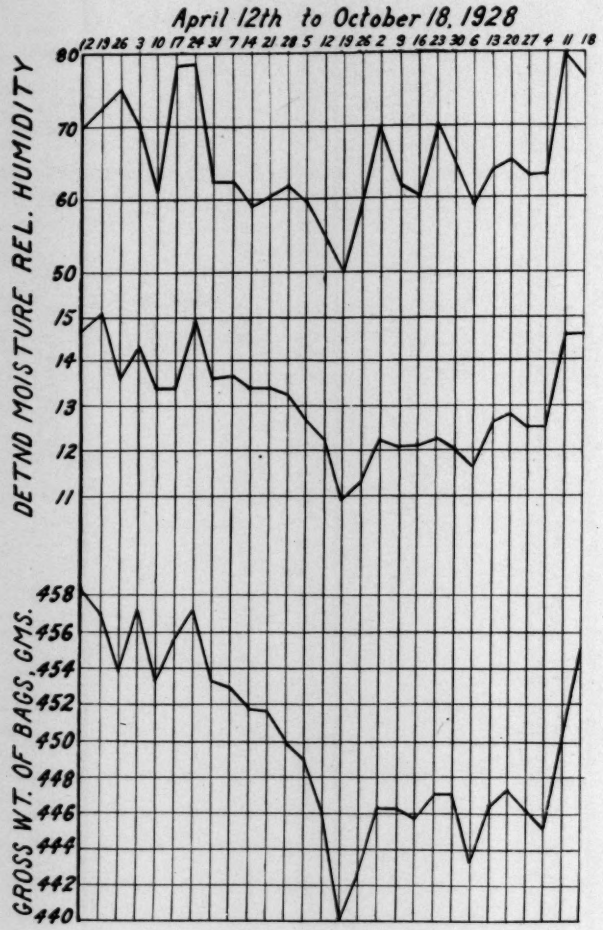

Fig. 3. Series 3. Exposure of One-Pound Paper Bags

humidities recorded at Rothamsted, and the broken line those obtained in the chamber. The bottom curve shows the variations in the weights of the bags.

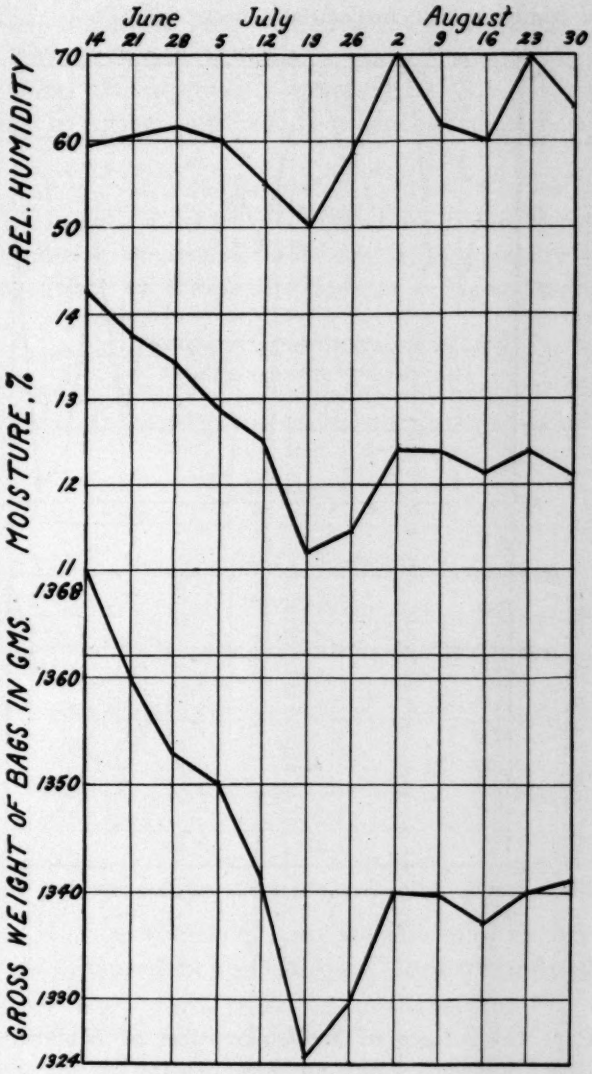

Fig. 4. Series 4. Exposure of Three-Pound Cotton Bags

From the curve it is seen that the Rothamsted humidities were closely followed, the slight discrepancies being due to admission of air of a different humidity when the cabinet was opened to weigh the bags.

These discrepancies about balance, some being slightly over and others slightly under the required humidity. The weight curve shows that there was an actual recorded loss of over 4%, slightly more than the greatest loss found by the inspector.

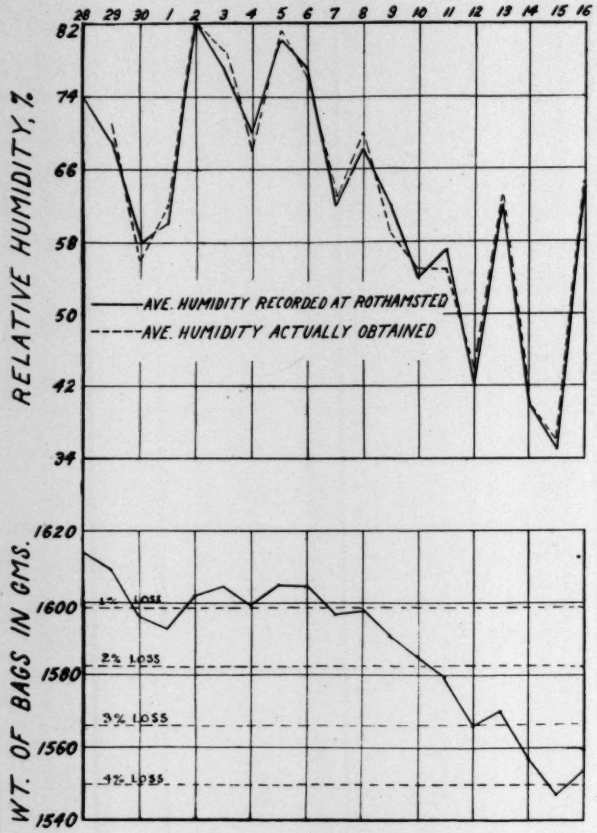

Fig. 5. Controlled Humidity Experiment

These curves were exhibited and explained in court by defending counsel (The Hon. Stafford Cripps K.C.) and the appeal was successful.

Part III. Rate of Re-Absorption of Moisture Lost by Evaporation

An experiment was carried out to learn how soon flour would recover the moisture lost by evaporation when resubmitted to the relative humidity with which it had been originally in equilibrium. A bag of flour weighing 693.3 gm. was subjected to a relative humidity of 75% for 24 hours and as there was no change in weight it was assumed that

the flour and the atmosphere were in equilibrium as regards moisture exchange. The relative humidity was brought down to 36% and after 24 hours a loss of 15.0 gm. was recorded. Then the relative humidity was increased rapidly to 75% and after 24 hours a gain of only 6.5 gm. was found. In the next 24 hours a further gain of 2.4 gm. occurred. After 120 hours the weight had increased by 11.3 gm. and remained constant for a further 192 hours, when the experiment was discontinued.

It thus appears that the whole of the loss is never recovered on re-exposure to the original relative humidity. In order to recover the original moisture content in equilibrium at 75% relative humidity it would be necessary to subject the flour to a relative humidity higher than 75%.

The results of this experiment are shown graphically in Figure 6. The broken line is the curve of the relative humidity, the full line is the curve of weight. It should be noted that the curve of relative humidity

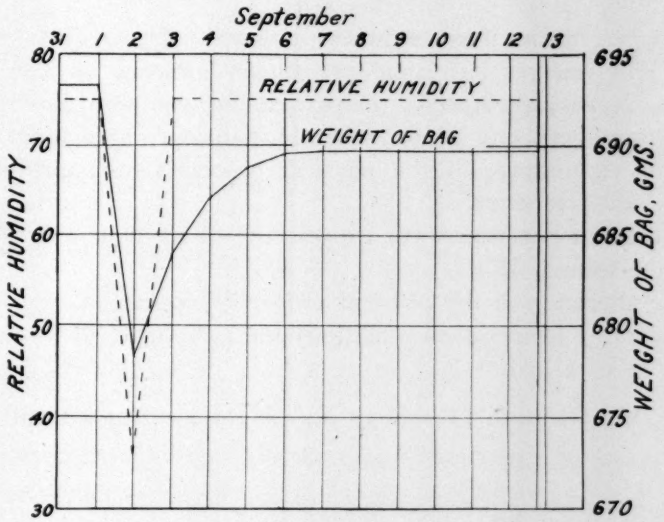

Fig. 6. Rate of Reabsorption of Moisture After Evaporation

was not built up from a series of averages, but the relative humidity was maintained at 75%, as indicated by the automatic self-recording hygrometer.

This experiment has been confirmed by a second exposure and after 17 days exposure to the original relative humidity of 75% the weight lost in one night at 40% R. H. had not been recovered (Fig. 7).

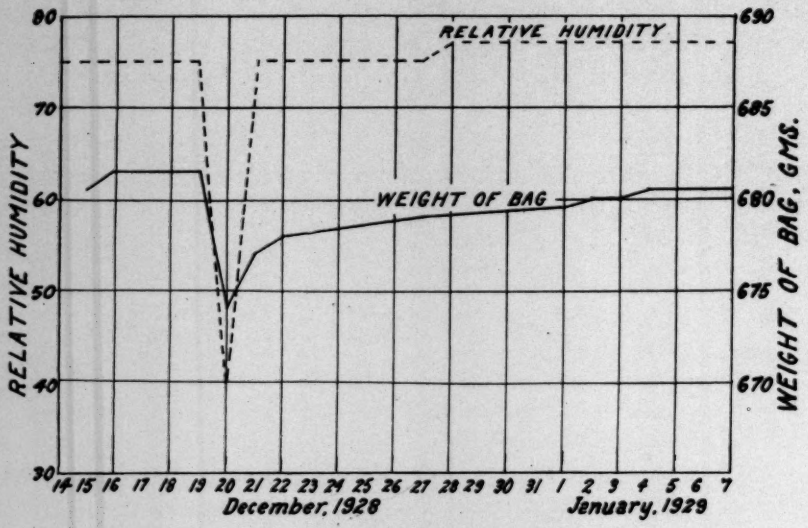

Fig. 7. Rate of Reabsorption of Moisture After Evaporation

It appears from these experiments that the drying of flour even at normal temperatures has some permanent influence on the colloidal particles. Apparently some of the particles become permanent gels, and the hydration capacity is permanently reduced. This may have an important significance in the physical processes of improvement of flour by heat treatment.

In the first experiment the temperature of the flour averaged 27°C. and in the second, 17°C.

The alteration in the physical properties appears, therefore, to be independent of temperature conditions and is brought about entirely by the process of dehydration.

Part IV. Relative Humidity and Moisture Content of Flour

A series of experiments was carried out to learn approximately at what relative humidity a flour of given moisture content will neither gain nor lose in weight.

The method adopted was to expose three tins accurately weighed and each containing 10 gm. of flour to varying humidities in the humidity chamber. The original moisture content of the flour was 13.06% and from the gain or loss recorded it was possible to calculate the moisture content of the flour at any given time during the experiment. The flour was exposed at various relative humidities, a series of weighings was taken, and the average variation in weight of the three tins used for calculations was recorded. In this way it was

possible to correlate moisture content with relative humidity and a curve has been plotted. The results obtained are recorded in Table II, and plotted graphically in Figure 8.

TABLE II

RELATION BETWEEN MOISTURE CONTENT OF FLOUR AND RELATIVE HUMIDITY, INITIAL MOISTURE 13.06 PER CENT

Average weight of tins and 10 gm. of flour	Gain or loss from original	Final moisture	Relative humidity, approx.
gm.	%	%	%
25.8746			
25.8654	—0.092	12.97	62
25.8766	+0.02	13.08	62
25.8606	—0.14	12.92	62
25.8550	—0.196	12.86	62
25.8717	—0.031	13.03	62
26.0913	+2.17	15.23	80
26.1371	+2.63	15.69	80
26.1340	+2.59	15.65	80
26.1515	+2.77	15.83	82
26.1582	+2.84	15.90	85
26.1458	+2.71	15.77	82
26.1818	+3.07	16.13	85
25.5089	—3.66	9.40	30
25.5320	—3.43	9.63	35
25.1073	—7.67	5.39	15
25.0240	—8.51	4.55	12
25.0007	—8.74	4.32	10
25.0273	—8.47	4.59	12
24.9893	—8.85	4.21	10
25.5399	—3.35	9.71	40
25.6427	—2.32	10.74	50
25.6620	—2.13	10.93	52
25.6693	—2.05	11.01	54
25.6640	—2.11	10.95	54
25.6517	—2.23	10.83	52
25.4735	—4.01	9.05	40
25.4419	—4.33	8.73	36
26.2367	+3.62	16.68	90
26.6223	+7.48	20.54	95
26.7506	+8.82	21.88	98

From the results obtained it appears that there is a close relation between the moisture flour can contain and the relative humidity of the atmosphere to which it is exposed, in other words, there is a definite moisture content of flour for any given humidity. The equilibrium ultimately attained is independent of the initial moisture content. When the relative humidity is about 10% the moisture content of flour settles to about 4%. When the humidity is increased to between 30% and 40%, the moisture rises to about 9.5%, and at 50% humidity the moisture reaches 10.75%. At a humidity of 90% the moisture content is nearly 17%; above this humidity, moisture content increases rapidly.

Between the humidities of 15% and 90% the moisture content of flour is a function of the relative humidity, and the relationship can be

plotted on a straight line. Beyond these limits the character of the relationship changes and above 90% there appears to be a continuous in-

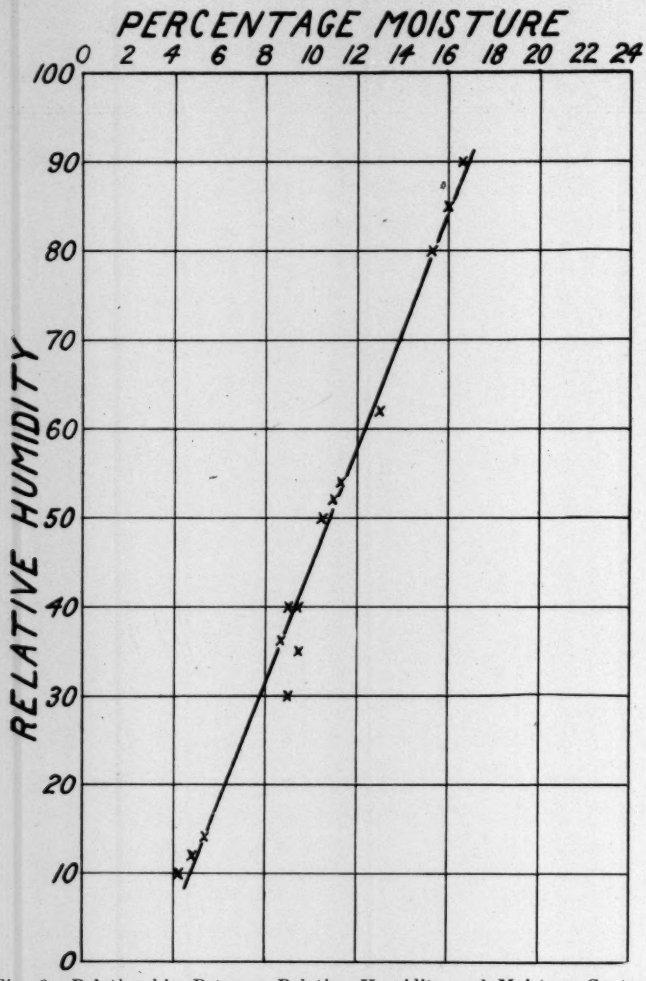

Fig. 8. Relationship Between Relative Humidity and Moisture Content

crease in moisture content, but the rate of increase decreases with increasing exposure of the flour, the curve thus becoming hyperbolic.

Part V. Moisture Exchange in Mixed Wheats

For a long time the practice of mixing a wet wheat with a dry wheat before conditioning has been common among English millers. This process has been known to bring about an exchange of moisture, the wet wheat becoming drier and *vice versa*. Very little seems to have

been published about the extent of the interchange or the time necessary for completion of interchange. Some millers allow the wheats to lie for as long as 14 days and believe that equality of moisture is reached between the two wheats, whereas others allow only a short time.

A series of tests was carried out in the laboratory to investigate the interchange between several pairs of wheats. The following pairs have been tested:

1. German 50%	Northern Spring Pacific 50%
2. German 50%	Australian 50%
3. English 50%	Australian 50%
4. English 50%	Plate (Baril) 50%
5. English 50%	Karachi 50%

Two methods have been adopted. In the first the wheats were well mixed and then allowed to stand in 100 cc. Nesslers, which were closed by tight corks. A corresponding set was carried out in open Nesslers. In the second set, flasks were used, both open and closed. It was found that there was very little difference whether the flasks were open to the atmosphere or closed.

Separation of the Wheats

At first an attempt was made to hand-pick the wheats, but this took a long time and also in the case of German and Northern Springs Pacific it was difficult to differentiate between some of the berries. It was decided, therefore, to make a mechanical separation. The German on the whole was much plumper than the Northern Springs Pacific and accordingly the German was passed over a sieve and the overtails were used for the test. Of the Northern Springs Pacific the throughs from the same sieve were used. The moisture content of the two fractions of each kind of wheat varied but slightly—within the limits of experimental error they were identical.

German:	Bulk	17.21%
	Tails	17.28%
N. Springs Pacific	Bulk	12.07%
	Throughs	12.08%

This method was employed for the separation of all the pairs of wheat in the tests. The results are shown in Table III and graphically in Figures 9, 10, 11, 12, and 13.

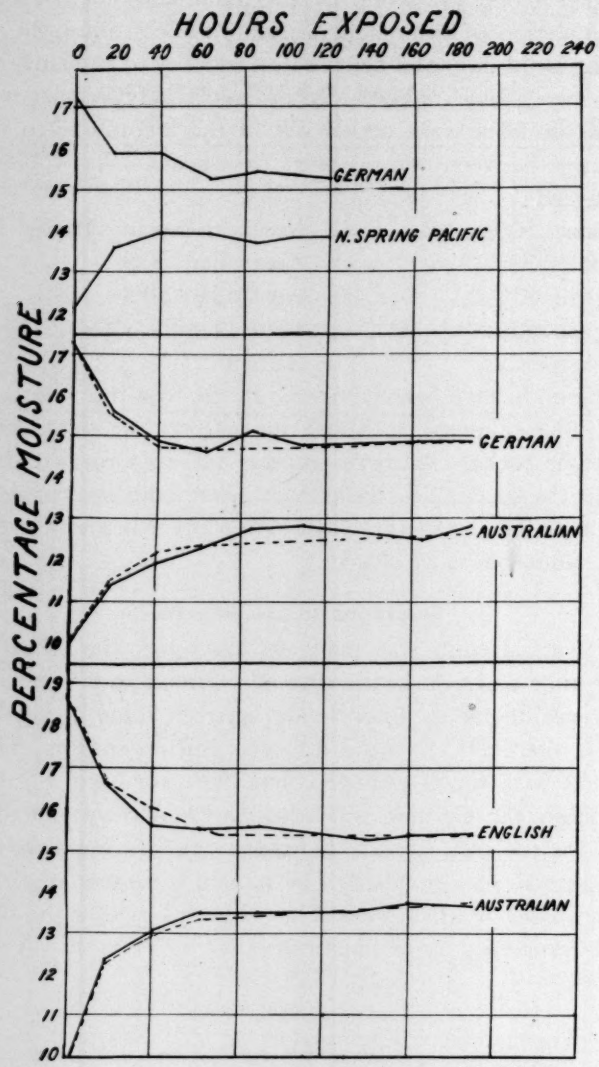

Fig. 9, 10, 11. Interchange of Moisture in Wheat

TABLE III

INTERCHANGE OF MOISTURE IN WHEAT

(a) German — Northern Springs Pacific (closed)								
	0	18	40	64	84	102	hours	
German	17.28	15.95	15.90	15.23	15.42	15.39	% moisture	
N. Springs Pacific	12.08	13.53	13.99	13.94	13.67	13.80	% moisture	
(b) German — Australian (closed)								
	0	18	40	64	84	112	162	186
German	17.33	15.64	14.85	14.60	15.07	14.75	14.88	14.79
Australian	10.07	11.38	11.87	12.27	12.70	12.84	12.38	12.71
(open)								
German	17.33	15.59	14.76	14.71	.....	.....	.....	14.80
Australian	10.07	11.48	12.15	12.30	.....	.....	.....	12.56
(c) English — Australian (closed)								
	0	18	42	66	90	138	162	186
English	18.53	16.59	15.69	15.52	15.58	15.27	15.44	15.45
Australian	9.76	12.37	13.02	13.48	13.52	13.51	13.77	13.65
(open)								
English	18.53	16.67	16.00	15.34	.....	.....	.....	15.44
Australian	9.76	12.27	12.99	13.27	.....	.....	.....	13.72
(d) English — Plate (closed)								
	0	16	40	64	88	136	160	.....
English	18.30	17.02	17.08	16.68	16.84	16.94	16.86	.....
Plate	13.62	14.62	14.81	14.61	14.95	15.00	14.94	.....
(e) English — Karachi								
	0	18	42	66	90	162	186	216
English	18.41	17.43	17.15	16.85	17.14	16.78	16.50	16.80
Karachi	12.68	13.55	13.83	14.31	14.32	14.42	14.15	14.40

Discussion

1. 50% German, 50% Northern Springs Pacific

The German fell from 17.28% to 15.90% in 40 hours and after 102 hours the moisture was 15.39%.

The Northern Springs rose from 12.08% to 13.99% in 40 hours and after 102 hours the moisture was 13.80%.

It is evident therefore that the main interchange was complete in 40 hours and equilibrium was reached with a difference in moisture between the wheats of 1.6%.

2. 50% German, 50% Australian

The German fell in 40 hours from 17.33% to 14.85% and after 186 hours the moisture was 14.79%.

The Australian rose from 10.07% to 11.87% in 40 hours but continued to gain in moisture up to 84 hours, when it reached 12.70% in spite of the fact that the German remained practically constant. In this case it appears that lying together for 3 days is necessary to gain the maximum interchange, and equilibrium is reached with a difference of 2.0% between the wheats.

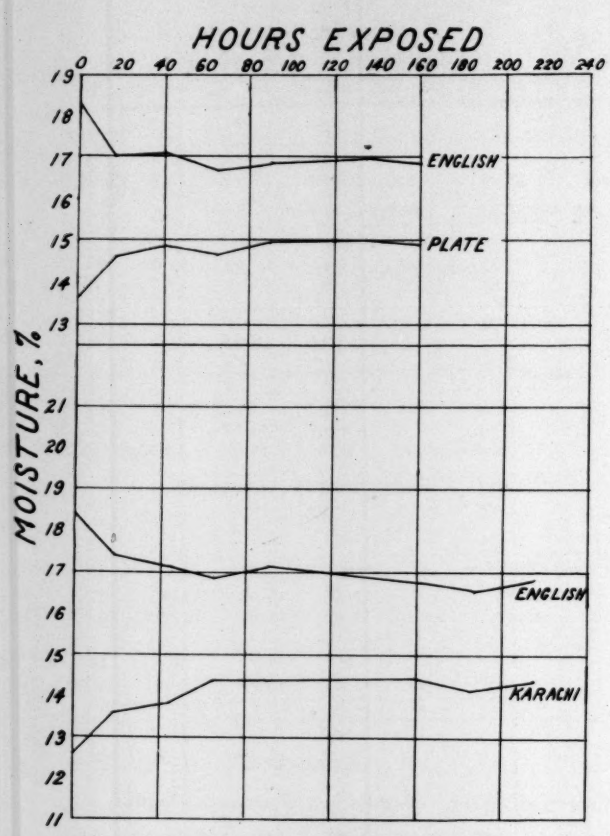

Fig. 12, 13. Interchange of Moisture in Wheat

3. 50% English, 50% Australian

The English started at 18.53% and fell to 15.69% after 40 hours. There was little further loss, the moisture after 186 hours being 18.45%.

The Australian started at 9.76% and in 40 hours had reached 13.02%, but there was a further rise of about 0.5% in the next 24 hours.

This is similar to the result obtained with German and Australian, in both cases the wet wheat reached its final moisture earlier than the dry one. It appears, therefore, that with Australian wheat and wet wheats like English or German the period for interchange should be 3 days, after which there is practically no further change. In this case the final moistures showed a difference of 1.8%.

4. 50% English, 50% Plate

The English started at 18.30% and in 40 hours had fallen to 17.08%. After 160 hours it was 16.86%.

The Plate rose from 13.62% to 14.81% in 40 hours and was 14.94% after 160 hours.

In this case the interchange was complete after 40 hours and the difference at the end was 1.9%.

5. 50% English, 50% Karachi

The moisture of the English fell in 66 hours from 18.41% to 16.85% and after 216 hours was 16.80%. The Karachi rose from 12.68% to 14.31% in 66 hours and then remained practically constant, being 14.40% after 216 hours. The interchange is thus complete in 66 hours and there is a final difference in this case of 2.4%.

From these five sets of tests the general conclusion is that when wet and dry wheats are mixed in equal quantities and allowed to lie together, moisture is transferred from the wet to the dry. The transference is complete in 3 days. Equality of moisture is not attained, there being a final difference of about 2% between the wheats.

Summary

1. Bags of flour with an initial moisture content of 13% to 15% fluctuated in weight on being exposed to the air.

2. The fluctuations in weight corresponded directly with the moisture content of the flour and with the relative humidity of the atmosphere.

3. It was possible in a specially constructed chamber to imitate given atmospheric conditions and then to show that in a prosecution under the Sale of Food (Weights and Measures) Act the loss recorded was entirely due to evaporation.

4. It has been shown that flour does not recover all the moisture lost by evaporation when re-subjected to the original relative humidity.

5. A series of determinations was made to find out at what moisture content flour will be in equilibrium with given atmospheric humidities.

6. A series of tests was made to study the rate and extent of moisture interchange between wet and dry wheats.

The author expresses his thanks to Messrs. McDougalls Limited, in whose laboratories this work was carried out, for permission to publish these experiments; and acknowledges the assistance of R. J. Wood, who carried out the experimental work of Part 5.

MINERALS OF WHEAT
PART I. SULFUR AND CHLORINE

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(Received for publication June 3, 1929)

It is well known that at the temperatures ordinarily used in ashing cereals there is a loss of some of the minerals. It is a common practice, however, to disregard this fact. Many discussions on the mineral metabolism and the acidic and basic character of various foods are based solely on the calculation of an analysis of the minerals as found in the ash. Owing to the importance of the ash test as an indication of the grade of flour and the uniformity of milling operations, previous papers from this laboratory have been concerned principally with the composition and character of the ashes of wheat and wheat products (Sullivan and Near 1927a, 1927b, 1927c). Some of the minerals of wheat (phosphorus, calcium, magnesium, iron, manganese, copper, zinc, etc.) are not lost in the ashing process and from an ash analysis an accurate calculation can be made of the amount of these elements in the wheat itself. Other elements such as sulfur and chlorine are almost entirely volatilized upon ignition at even low temperatures and traces of these elements found in the ash give an entirely false idea of the amounts of sulfur and chlorine actually present in the product as it is used.

So far as the authors are aware no complete analyses of sulfur and chlorine have been recorded on a single wheat and the products milled from it. Osborne (1907) reported the ultimate analyses, including sulfur, of several protein fractions obtained from wheat. In some of the earlier German works and in a few textbooks on nutrition (Mentzel & Von Lengerke 1905, Albu & Neuberg 1906, Sherman 1919) tables compiled from various sources give the chlorine and sulfur analyses of bread, flour, and bran. No details as to the methods or analyses of the products, however, are given.

Experimental

A hard spring Marquis wheat grown in North Dakota and the short patent, clear, and bran obtained from milling it in a 3,000 barrel mill were taken for analysis. The bread was baked in a commercial bakeshop from the patent flour according to the following formula:

458 lb. flour	12.5 lb. lard
283 lb. water	9 lb. 12 oz. salt
7 lb. yeast	13 lb. sugar
18 oz. Arkady	26 lb. condensed milk (8% fat)
5 lb. malt extract	

Ash was obtained by ignition for 16 hours at 590°-600°C.

The determination of phosphorus, calcium, and magnesium was made on the same weighed sample of ash. After elimination of silica and carbon, phosphorus was determined according to the gravimetric pyrophosphate method. Calcium and magnesium were determined on the filtrate from the yellow precipitate, calcium by the volumetric permanganate method, and magnesium by the method of Schmitz (Treadwell Hall 1919). Phosphorus was also determined on the products directly by heating with concentrated sulfuric acid and a drop of mercury. These results checked very well with the phosphorus of the product as calculated from the ash, which showed that no phosphorus had volatilized. No loss would be expected as it was shown before (Sullivan and Near 1927c) that most of the phosphorus in wheat ash is present as pyrophosphate, which is alkaline. Losses upon ignition are more likely to affect the mineral acids than the mineral bases and the loss is largest in products that have a strongly acidic ash. The ash of wheat and its products is alkaline.

Calcium and magnesium were determined on the ash and calculated to the product, as no loss of these elements occurs in ashing. Sulfur was measured by heating 5 grams of sample with 50 cc. magnesium nitrate solution on an electric hot-plate and then in an electric muffle until no black particles remained, which is according to the tentative method of the A. O. A. C. (1925). The sample was then moistened with water and 30 cc. concentrated hydrochloric acid was added. The solution was heated to boiling, filtered, washed, and the sulfur precipitated with barium chloride. Blanks were determined on all reagents used. Sulfur was determined on the ash by dissolving the ash in dilute hydrochloric acid, filtering, and precipitating with barium chloride.

Chlorine was determined on the products by a procedure recommended by Tilden (1928). To 7 grams of sample contained in a platinum dish, 800 mmg. of c.p. sodium carbonate was added in solution. The mixture was evaporated and charred in a muffle at as low a temperature as was possible in order to get a white ash. This temperature varied considerably from the patent to the bran. The ash was dissolved in 50% nitric acid and chlorine was subsequently determined by the gravimetric procedure. The chlorine on the ash was measured

after removal of silica by dissolving the ash in nitric acid and precipitating directly with silver nitrate according to the gravimetric procedure.

Discussion

In Table I the analyses of wheat and the patent, clear, and bran obtained from milling the same wheat; and bread baked from the patent flour, are given. The percentages of phosphorus, calcium, and magnesium in the products, as calculated from the ash; and the chlorine and sulfur, as determined directly on the product, are reported. Sulfur and chlorine percentages in the bread are dependent on the amount of salt and yeast food used in the bread formula. All results are calculated to dry basis. In Table II partial analyses of the ashes from all products are given. Chlorine was determined on the ash and found in very small amounts. Using a one-gram sample of ash for the sulfur determination, the precipitate formed by barium chloride was too small to weigh.

TABLE I

ANALYSES OF MARQUIS WHEAT AND ITS PRODUCTS

(Calculated to dry basis.)

	Wheat	Patent flour	Clear flour	Bran	Bread
	%	%	%	%	%
Lipoid	3.17	1.69	2.47	6.19	4.41
Protein*	15.39	13.30	16.79	19.52	13.15
Ash	1.857	0.453	0.778	7.177	2.769
Magnesium	0.1725	0.0287	0.0677	0.7234	0.0399
Calcium	0.0475	0.0155	0.0254	0.1220	0.0800
Phosphorus	0.4282	0.1130	0.1918	1.6334	0.1400
Chlorine	0.0546	0.0507	0.0601	0.0387	1.0053
Sulfur	0.1943	0.1648	0.2104	0.2547	0.1923

* Protein factor, N x 5.7, used for wheat, patent, and clear; protein factor, N x 6.25, used for bran and bread.

TABLE II

ANALYSES OF THE ASHES OF MARQUIS WHEAT AND ITS PRODUCTS

	Wheat	Patent flour	Clear flour	Bran	Bread
	%	%	%	%	%
Magnesium	9.29	6.33	8.71	10.08	1.44
Calcium	2.56	3.42	3.26	1.70	2.89
Phosphorus	23.06	24.95	24.65	22.76	4.91
Chlorine	0.064	0.050	0.033	0.035	1.062
Sulfur	Trace	Trace	Trace	Trace	Trace

Table III shows the percentage of chlorine in the products as calculated from the percentage found in the ash, showing a loss of from 93% to 99% of chlorine due to ignition. The chlorine percentages of the samples were about the same for all the grades.

TABLE III

CHLORINE CONTENT OF MARQUIS WHEAT AND ITS PRODUCTS

	Chlorine as found in product	Chlorine in product calculated from chlorine found in ash	Chlorine lost upon ignition
	%	%	%
Wheat	0.0546	0.0012	97.8
Patent	0.0507	0.0002	99.6
Clear	0.0601	0.0003	99.5
Bran	0.0387	0.0025	93.5
Bread	1.0053	0.0294	97.1

It is interesting to note that as the protein in the various products milled from a wheat increases the sulfur content likewise increases. In Table IV the percentage of sulfur in the protein is fairly constant for the patent, wheat, clear, and bran. Practically all the sulfur of wheat is held as a component of cystine,

$$\begin{array}{ll} CH_2 - S - S - CH_2 \\ CH.NH_2 \qquad CH.NH_2 \\ COOH \qquad COOH \end{array}$$

one of the amino acids obtained in small amounts from the hydrolysis of the gliadin, glutenin, leucosin, and globulin of wheat.

Sodium and potassium will be discussed in a later article.

TABLE IV

SULFUR AND PROTEIN CONTENTS OF MARQUIS WHEAT AND ITS PRODUCTS

	Protein	Sulfur	Sulfur in total protein
	%	%	%
Patent	13.30	0.1648	1.24
Wheat	15.39	0.1943	1.26
Clear	16.79	0.2104	1.25
Bran	19.52*	0.2547	1.29
Clear gluten	86.25	0.9286	1.08
Patent gluten	87.50	0.9724	1.11

* N x 6.25.

Summary

1. Determinations of sulfur and chlorine were made on a wheat and products milled from it and also on bread baked from the patent flour.

2. The higher the protein content of the samples analyzed the higher the sulfur content. Sulfur is combined organically as a component of cystine.

3. Sulfur and chlorine were almost completely lost upon ignition of samples at 590°-600°C.

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PLASTICITY OF FLOUR-WATER SUSPENSIONS[1]

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Sharp and Gortner (1923), in the work on the viscosity of extracted flour-water suspensions that had been acidulated, aroused much interest and initiated a large amount of experimental work on viscosity and its relation to the baking quality of flour. They review fully the earlier work on viscosity. They recognized that some of their suspensions were plastic, and later Sharp (1926) studied the plasticity of certain flour-water suspensions.

Sharp and Gortner found that a number of factors influenced the viscosity of flour-water suspensions, the most important of which were hydrogen-ion concentration, presence of electrolytes, and concentration of the suspension. They related the tangent of the angle (constant b) formed by plotting the log of the viscosity against the log of the concentration of the suspension to the strength of flours.

1 Published with the approval of the director as Scientific Paper No. 159, College of Agriculture and Experiment Station, State College of Washington.

These viscosity measurements were made on flours that had been adjusted to a H-ion concentration equivalent to a pH of 3.0 or above. Doughs brought to this pH have lost their baking strength. They believe that glutenin is the protein mainly responsible for the marked imbibitional power of flour.

Johnson (1927) calls attention to the difficulty of duplicating results on the same flour over a period of time. He found that extraction at higher temperatures caused greater viscosity owing to a more complete removal of electrolytes. Greater agitation in preparing the suspensions produced higher viscosity, because the electrolytes were more completely removed. He notes that hydration is a function of time, but does not consider it an important factor after one hour and before twenty-four hours. Johnson and Herrington (1928) note that a large percentage of the total nitrogen of flour is removed by extraction. This removal of nitrogen probably contributes to a decrease in viscosity. They suggest that as the H-ion concentration of flour increases during storage, the viscosity might be expected to increase.

Denham, Blair, and Watts (1927) found that when the decrease in viscosity noted by Sharp and Gortner with the Ostwald viscometer was extrapolated to zero time it was a constant for any given flour and partially, at least, correlated with the strength of that flour. They believe it possible to use capillary tubes of comparatively large bore, as the flow was probably not turbulent with a substance as viscous as a flour suspension. They used tubes with bore from 0.138 to 0.496 cm. They believe that the decrease in viscosity, or aging of a flour suspension, is probably a case of syneresis. Hatschek agreed that the changes were probably of syneretic origin. Van der Lee (1928) thinks that most of the work that has been done on the viscosity of flour suspensions is empirical.

Smith (1925), in a study of the relation of viscosity to baking quality, concludes that viscosities determined by leaching out the soluble constituents do not seem to have any more merit in indicating baking quality than do the viscosities determined upon the flour without leaching. Blish and Sandstedt (1925) said that since the electrolytes are natural flour constituents and are present in bread dough, they should also enter into viscosity determinations; also that the value of viscosity data was slightly lowered rather than improved by preliminary removal of the electrolytes. They believe this would indicate that the preliminary removal of electrolytes is unnecessary when flours of similar ash content are to be compared with each other. Sharp (1926) found that a particular flour showed plastic properties in a

suspension containing 19 per cent or more of flour by dry weight. He used a vertical type of plastometer. The radius of his capillary tubes was 0.0793 cm. Even with a capillary as large as this he had difficulty in obtaining concordant results on duplicate suspensions. His suspensions were prepared by shaking for 30 minutes. The suspensions were not extracted or acidified. He does not state the pressure used. The time factor was apparently not considered.

Experimental

The plastometer and the method used were partially described by St. John (1927), and by St. John and Bailey (1929). A temperature of 25°C. was maintained by a Freas large-size sensitive water thermostat. A high-grade split-second stop watch was used for timing. A water manometer was used to determine the pressure, which was maintained at 40-41 cm. or 60-61 cm. The work reported was done with three different capillary tubes, No. 5 having an average radius of 0.1217 cm., No. 10 an average radius of 0.1233 cm., and No. 12 an average radius of 0.1334 cm. The working constants for tubes 10 and 12 were

	Tube 10		Tube 12	
Section	$\triangle v$	$\frac{\pi r^4 g}{8\left(\frac{l_2 + l_1}{2}\right)}$	$\triangle v$	$\frac{\pi r^4 g}{8\left(\frac{l_2 + l_1}{2}\right)}$
	cc.		cc.	
4	0.4681	0.002568	0.5693	0.003389
5	.4781	.001992	.5701	.002671
6	.4911	.001642	.5690	.002203
7	0.4976	0.001402	0.5713	0.001878

$\triangle v$ was divided by the time of flow for each section of the tube as determined with the stop watch, giving the rate of flow $\triangle v / \triangle t$. This was plotted against the shearing force in dynes per square centimeter obtained by multiplying the observed pressure by $\frac{\pi r^4 g}{8\left(\frac{l_2 + l_1}{2}\right)}$ The tangent of the angle thus formed is defined as the mobility of the substance and is calculated by the method of least squares. The yield value is also calculated by the method of least squares.

Measurements of plasticity were made 30 and 60 minutes after the water was poured upon the flour. The flours were not extracted and the suspensions were not acidified. Suspensions were prepared in duplicate in each case, the second being mixed 15 minutes after the first.

The suspensions were prepared by adding the exact amount of water to the weighed sample of flour in a beaker and stirring gently

with a spatula. A small portion of this mixture was poured into a small mortar (100 mm.) and worked gently, holding the pestle as one holds a pencil. This was poured out and successive portions of the mixture were worked into a smooth suspension. This procedure requires about 10 minutes. This method of preparing the suspensions avoids any violent treatment that might affect the results in such a colloidal suspension and also avoids the production of foam, which occurs during shaking with the consequent danger of affecting results. It also allows the making of the first measurement at the end of a minimum period of time. The results obtained with this method were more satisfactory than those obtained when the suspensions were shaken. In fact, the preparation of suspensions by shaking was early abandoned because of irregular results.

Ten different flours were used during the course of this work. Flours 1, 2, 3, 6, and 7 were milled from Minnesota wheats by the State Experimental Mill in Minneapolis.[2] Flours 1, 2, and 3 were different lots of spring wheat patents milled at different times. Flour 5 was a Montana wheat containing an exceptionally large amount of protein. Flour 6 was a Minnesota wheat, above the average of the Minnesota crop. Flour 7 was a soft wheat grown south of Minneapolis. Flour 4 was a cracker flour of unknown history. Flours 8, 9, and 10 were from Washington wheat.[3] Analytical data on the flours used are given in Table I.

TABLE I

ANALYTICAL DATA ON FLOURS USED

Flour No.	pH. (1-6-28)	Loaf volume	Crude protein	Ash	Original moisture	Moisture (12-28-27)	Date milled
		cc.	%	%	%	%	
1	5.71	2120	12.10	0.49	12.95	9.00	11- 1-25
2	5.78	2200	12.70	0.50	13.60	8.82	1- 6-26
3	5.66	2110	11.50	0.43	13.81	9.95	6- 1-26
4	5.58	....	11.18	....	.....	9.29	.....
5	5.70	2575	17.84	0.55	13.21	8.85	9- 6-26
6	5.65	2725	13.57	0.47	14.49	9.16	9- 6-26
7	5.77	2250	9.58	0.44	15.38	9.38	9- 6-26
8*	5.33	2420	11.70	0.49	12.80	7.75	4-30-27
9†	5.29	2310	11.70	0.50	12.40	7.90	4-30-27
10‡	5.29	2230	11.80	0.50	12.30	7.78	4-30-27

* W. Marquis 25% Baart 75%
† W. Marquis 25% W. Turkey 25% Baart 50%
‡ Baart 80% Burbank 20% Fortyfold 20%

Previous to a study of the significance of the plasticity data we have considered the variation between the measurements on duplicate sus-

2 We are glad to express our thanks to Dr. R. C. Sherwood for preparing these flours and for a part of the analytical data contained in Table I.

3 We are grateful to T. R. James, of the Sperry Flour Company, Spokane, for the Washington flours and analytical data.

pensions. Taking 260 representative measurements we find that the average difference between duplicates is 0.0542. This mean includes measurements made after 30 and after 60 minutes. The mean of the difference between duplicates on measurements made after 30 minutes is 0.0553, and after 60 minutes is 0.0532.

A limited number of measurements was given in the article by St. John (1927) to show the similarity of results obtained with the plastometer there described and the Bingham and Murray instrument. The readings given in that paper were made 60 minutes after the flour and water were mixed. Additional data are given in Table II, showing measurements made after 30, 60, and 90 minutes with tubes 5 and 10, whose dimensions are given earlier. Flours 1, 3, and 4 were used.

TABLE II

MOBILITY OF SUSPENSIONS

Flour No.	Time after mixing min.	Tube No. 5				Tube No. 10			
		1	2	3	Av.	1	2	3	Av.
1	30	0.589	0.665	0.663	0.639	.....	0.635	0.640	0.638
	60	0.677	0.732	0.761	0.723	.....	0.740	0.755	0.748
	90	0.701	0.784	0.827	0.771	0.760	0.720	0.716	0.732
3	30	0.582	0.598	0.592	0.591	0.630	0.685	0.629	0.648
	60	0.759	0.714	0.780	0.751	0.731	0.742	0.777	0.750
	90	0.833	0.799	0.819	0.817	0.761	0.751	0.811	0.774
4	30	0.647	0.641	0.594	0.627	0.575	0.673	0.582	0.610
	60	0.593	0.648	0.688	0.643	0.667	0.793	0.693	0.717
	90	0.671	0.703	0.725	0.700	0.728	0.766	0.768	0.754

It appears from these data that not only may measurements be duplicated with a very satisfactory degree of accuracy for biological materials but that such duplication of results may be obtained with different sets of apparatus. The pressure used for these measurements was 40-41 cm. of water.

Another result evident from these data as well as from all other measurements made is a slight but persistent tendency for the mobility to increase as a given suspension stands. This observed tendency confirms the observation made by St. John and Bailey (1929) and is undoubtedly similar to the decrease in viscosity noted by Sharp and Gortner (1923). This phenomenon is thought by Denham, Blair and Watts (1927) and by Hatschek to be a case of syneresis. In order to study the extent of this phenomenon over a longer time, measurements were made on four flours over a period of 7 or 8 hours. These results are shown in Table III.

TABLE III

INCREASE OF MOBILITY OF FLOUR SUSPENSIONS WITH TIME

Hours after mixing	Flour 6		Flour 7		Flour 8		Flour 10	
1	0.393	0.352	0.818	0.937	0.444	0.499	0.443	0.426
2	0.611	0.457	0.829	.....	0.514	0.517	0.674	0.611
3	0.504	0.468	0.779	0.717	.....	.....	.....	.....
4	.....	.....	.....	.....	0.737	0.619	0.562	0.742
5	0.664	0.546	1.267	1.034	0.639	0.660	0.666	0.780
6	0.561	0.581	1.001	1.035	0.804	0.643	0.800	0.799
7	0.645	0.489	1.089	1.403	0.673	0.624	0.930	0.734
8	0.756	0.878	0.973	1.164	.....	.....	.....	.....

It appears from these data that the mobility continues to increase gradually altho not entirely regularly throughout 7 or 8 hours.

TABLE IV

EFFECT OF CONCENTRATION ON MOBILITY

Flour No.	Min. after mix.	Concentration of flour in suspension, per cent										
		30.5	30.0	29.7	29.6	29.3	29.0	28.7	28.4	28.3	28.2	25.9
1	30	.....	0.452	0.507	.....	.....	0.548	0.584	.....	.....	.....	.....
	60	.....	0.506	0.513	.....	.....	0.540	0.646	.....	.....	.....	.....
3	30	0.387	.....	.....	.....	.....	.....	0.560	.....	.....	.....	.....
	60	0.424	.....	.....	.....	.....	.....	0.540	.....	.....	.....	.....
4	30	.....	0.543	0.586	.....	.....	.....	.....	.....	.....	.....	.....
	60	.....	0.565	0.550	.....	.....	.....	.....	.....	.....	.....	.....
5	30	.....	0.188	.....	0.232	.....	.....	.....	.....	.....	.....	.....
	60	.....	0.222	.....	0.237	.....	.....	.....	.....	.....	.....	.....
6	30	.....	0.370	.....	.....	.....	.....	.....	.....	.....	0.606	0.838
	60	.....	0.418	.....	.....	.....	.....	.....	.....	.....	.....	0.868
8	30	.....	0.240	.....	.....	0.294	.....	.....	0.417	.....	.....	.....
	60	.....	0.280	.....	.....	0.332	.....	.....	0.532	.....	.....	.....
10	30	.....	0.371	.....	.....	.....	.....	.....	.....	0.400	.....	.....
	60	.....	0.466	.....	.....	.....	.....	.....	.....	0.502	.....	.....

Table IV shows the extent of the variation of mobility with change in concentration of the flour suspension. The increase in the mobility with the decrease in concentration is of the general order of 0.10 for each decrease of 1 per cent in concentration at the concentrations used.

Care has been used in the work reported here to keep the pressures within comparatively narrow limits. To study any variation in mobility that might be caused by variation in pressure, measurements were made at different pressures, using two flours as shown in Table V.

These data show a slight but consistent increase in mobility where higher pressures are used. The amount of this variation is, in general, less than the average difference between duplicate measurements, so that for the pressures used these differences have little significance.

A question arises regarding the variation caused by small differences in the pressure reading and in reading the time of flow. An

error of 0.1 cm. in reading the pressure on the water manometer would cause an error of only 0.0006 in the mobility value; the maximum difference due to an error of 0.1 second in reading the time would be 0.0075.

TABLE V

EFFECT OF VARYING PRESSURE ON MOBILITY

Pressure, cm. water	Minutes after mixing	Mobility				Average
		Flour No. 6				
	30	0.303	0.361	0.441	0.371	0.369
60	60	0.412	0.401	0.398	0.390	0.400
	30	0.355	0.453	0.395	0.531	0.433
120	60	0.482	0.520	0.555	0.747	0.576
		Flour No. 7				
	30	0.662	0.651	.....	.....	0.657
40	60	0.719	0.721	.....	.....	0.720
	30	0.731	0.596	0.701	0.747	0.694
60	60	0.769	0.809	0.663	0.816	0.764

Experience indicates that the total time of flow throughout the four 10-cm. sections of the tube should preferably be between 45 and 60 seconds to give the desired type of flow with these suspensions. Conditions should be adjusted to give this rate of flow.

Flours are known to change with age, especially in baking quality and hydrogen-ion concentration. Data are tabulated in Table VI to show any "ageing" which these flours may have undergone in mobility during the course of this study.

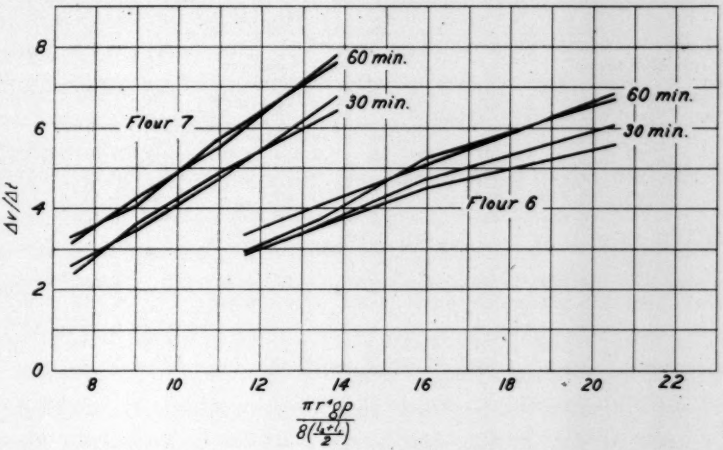
TABLE VI

EFFECT OF AGE OF FLOUR ON MOBILITY

Date of measurement	Minutes after mixing	Flour No.						
		1	2	3	4	5	6	7
10-23-26	30	0.638	0.690	0.620	0.619	0.408	.....	.....
	60	0.733	0.737	0.751	0.680	0.531	.....	.....
3-15-27	30	0.655	0.695	0.653	0.659	0.369	.....	.....
	60	0.679	0.741	0.744	0.677	0.377	.....	.....
12-27-27	30	0.452	0.442	.....	.....	.....	0.370	0.760
	60	0.506	0.615	.....	.....	.....	0.418	0.733
4-13-28	30	.....	.....	.....	.....	.....	0.369	0.694
	60	.....	.....	.....	.....	.....	0.400	0.764

The data in Table VI indicate, with one possible exception, that there is no change in the mobility of these flours with age. Flour No. 1 was about a year old when the first measurement shown in Table VI was made; Flour No. 2 was 9½ months old, No. 3 was about 4½ months old, No. 4 about 2 years old, No. 5 about 1½ months old, and Nos. 6 and 7 about 14½ months old. The only part of the data

that suggests a change in mobility with the age of the flour is the measurements on flour No. 1 made when it was about 26 months old and on flour No. 2 when it was about 2 years old. Any ageing effect on mobility may, therefore, appear after the flours are past the age when they are ordinarily used for baking.



The figure shows curves plotted from data chosen at random from among the large number of measurements made. $\Delta v/\Delta t$ has been plotted against $\frac{\pi r^4 gP}{8\left(\frac{l_2+l_1}{2}\right)}$. Logarithms of the data have not been plotted. The curves represent duplicate measurements after 30 and after 60 minutes on flours No. 6 and No. 7. The points on the various curves appear to fall on a straight line. The change in mobility of the suspensions between 30 and 60 minutes after mixing is evident with both flours, the rate of flow increasing with the length of time the suspension stands. The tangents determined graphically and by the method of least squares agree closely.

In Table VII the mobility and other data have been arranged in the order of the baking quality of the different flours in order to show any possible correlation between mobility and baking quality as shown by loaf volume. It will be noted that the loaf volume of the nine flours varies through a rather wide range. The baking data were obtained in three different laboratories so that they are not strictly comparable. However, it is believed that they show the general relation between the different flours. The baking of Flours 1, 2, and 3 and of Flours 5, 6, and 7, altho done by different workers in different laboratories, was carried out by exactly the same method under strictly controlled conditions, using identically the same formula. The results of

the two laboratories are known to have duplicated closely. The results on Flours 8, 9, and 10 are believed to be comparable to the others. The baking quality of Flour 4, a cracker flour, is assumed to be lower than the others.

TABLE VII

RELATION BETWEEN MOBILITY AND LOAF VOLUME

Concentration of dry matter suspension, per cent	Minutes after mixing	Flour No. 6	5	8	9	7	10	2	1	3	4
		Loaf volume cc. 2725	2575	2420	2310	2250	2230	2200	2120	2110	....
						Mobility					
27	30	.....	0.232	0.294	.....	0.633	0.400	.....	0.507	0.523	0.586
	60	.....	0.237	0.332	.....	0.742	0.502	.....	0.513	0.504	0.550
27.25	30	0.370	0.188	.....	.....	0.760	.....	0.442	0.452	.....	0.543
	60	0.418	0.223	.....	.....	0.733	.....	0.615	0.506	.....	0.565
27.65	30	.....	.....	0.240	0.289	.....	0.371	.....	.....	.....	.....
	60	.....	.....	0.280	0.342	.....	0.466	.....	.....	.....	.....
11.7 per cent protein	30	0.838	0.254	0.240	0.289	0.182	0.371	.....	0.548	0.387	.....
	60	0.868	0.353	0.280	0.342	0.207	0.466	.....	0.540	0.424	.....
pH.		5.65	5.70	5.33	5.29	5.77	5.29	5.78	5.71	5.66	5.58
Protein, per cent		13.57	17.84	11.70	11.70	9.58	11.8	12.70	12.10	11.50	11.18

If we consider the mobility for flours 5, 8, 10, 1, 3, and 4, where 27 per cent of dry matter was present in the suspensions, we note a progressive increase in the mobility as the volume of bread produced from these flours decreases. Flours 1 and 3 produced loaves of practically the same volume and the mobility values are very nearly the same. Flour 7 does not fall into this sequence. Other measurements at a slightly different concentration show a similar sequence for Flours 5, 2, 1, and 4. Flour 7 is again out of line, and Flour 6 does not fit into the series. At a third concentration we find that Flours 8, 9, and 10 show clearly this decrease in mobility (increase in plasticity) with increase in loaf volume.

Another series of measurements was made, using suspensions adjusted to contain equal amounts of protein equivalent to flours 8 and 9, containing 11.7 per cent protein. No correlation appears between these mobility values and loaf volume. This suggests that other factors besides the quality of protein, as indicated by this general type of measurement, are active in determining the quality of the bread produced.

The above measurements also emphasize the hazard of drawing conclusions from measurements on two or three flours. For instance, using only Flours 8, 9, and 10 the conclusion might be drawn that, as the flours contain equal quantities of protein, the apparent correlation of the measurements was due to differences indicated in the quality of the protein, on the basis of Sharp and Gortner's (1923) work, by

these measurements of mobility. On the basis of such measurements on the eight flours such a conclusion is apparently not warranted.

It is apparent that in this series of ten flours there is no correlation between loaf volume and the percentage of protein present or the H-ion concentration.

The possibility of a closer correlation between loaf volume and two of these factors or all three (mobility, H-ion concentration, and percentage of protein) was briefly investigated by some simple mathematical calculations. No correlation appears between loaf volume and the two factors, mobility and H-ion concentration, or between volume and all three factors.

The yield values have been calculated for a large number of the measurements. Those corresponding to the data presented in Table VII were tabulated. The values vary between 0.01 and 0.09. The average difference between duplicates is 0.011. There seemed to be no correlation between yield values and any other factors.

Summary

A method of measuring the plasticity of flour-water suspensions has been studied. Measurements, expressed in terms of mobility, show that results may be closely duplicated. An increase of mobility is evident as a suspension stands. The time factor must be considered in making these measurements. Mobility varies with the concentration of the suspension. The mobility varied only slightly with the pressures used. The mobility may be determined from the measurements either graphically or by calculation.

A change in mobility due to ageing of the flours is not shown by these results. Eight of the flours used show an increase in plasticity from flour to flour as the loaf volume increases. The yield values for all the flours are practically the same.

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INFLUENCE OF FLOUR MOISTURE ON THE PEKAR TEST

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(Received for publication May, 1929.)

While the Pekar test is possibly of little scientific interest, references to it are to be found in the literature of cereal chemistry and a description of the test is given in the A.A.C.C. Book of Methods. The following observations in connection with this method of determining flour color may not, therefore, be without some interest, especially as the test is so widely used in commercial laboratories and in mills.

It has been noticed that flour kept in the laboratory during winter, when the relative humidity of the air often drops below 35%, rapidly becomes whiter (as judged by 'slick appearance') if it is stored in sacks, or in containers with loosely fitting covers. Associated with this change in color there was a lowering in the flour moisture. Samples kept under similar conditions, but in containers that prevented the loss of moisture, remained relatively creamy. A discrepancy between the Pekar color and the color of the bread was also noticed. The low-moisture samples produced bread of poorer color than would be expected from their appearance on the slick. Incidentally, the change in slick appearance gave rise to practical difficulties, since samples kept for a short time in the laboratory differed so much from freshly milled flour as to render them valueless as standards in making comparative tests for color.

A sample of unbleached flour (ash 0.40%, protein 11.0%) was divided into two portions. One was exposed to the dry air of a desiccator; the other was placed in a similar desiccator containing water instead of sulphuric acid. At the end of 22 hours the two portions, now containing 10.9% and 15.1% of moisture, were slicked against each other. When the dipped slick was dried in the usual way it was found that the drier flour appeared much the whiter. After 46 hours, when the samples contained 9.2% and 15.6% moisture, the difference in Pekar color was even more pronounced, the drier portion having the appearance of a heavily bleached flour. No difference, however, could be detected in the gasoline color of the two portions.

The flour containing 9.2% moisture was next exposed to moist air at room temperature and thus brought back to its original moisture content. It was found to be indistinguishable on the slick from the original flour.

In another experiment the moisture content of the flour was reduced to 1% by drying in a vacuum desiccator. On the slick it looked very white as compared with the decidedly creamy color of the original, but when its moisture content was raised again by exposure to humid air, the flour returned to its original color.

Similar experiments have been made with many samples of different grades of flour milled from Canadian spring wheat, with flours milled on an experimental mill from durum wheats, and with flours from Ontario and Pacific Coast winter wheats. Whether bleached or unbleached samples were used, it was found that as they lost moisture through exposure to dry air at room temperature they became whiter by the Pekar test, and also that the original color could be restored by exposure to moist air, thus returning the lost moisture.

What is judged in the Pekar test is not the color of the flour, but of the partly transparent covering of dried dough on a background of flour. When high and low moisture flours were slicked against each other it was observed, during the drying in the oven, that the dough over the high-moisture flour remained soft after that covering the low-moisture portion had become dry and brittle. Also, when it did dry the dough was more horny and less easily broken than that on top of the dry flour.

It was first thought that these observations pointed to some difference in the structure of the doughs formed by dipping flours of varying moisture content, and that these differences would disappear if the flours were allowed to remain immersed in water for a longer time in order to give the dry flour an opportunity to imbibe water. The differ-

ences in slick color did become less as the time of immersion was increased, but when flours containing 14.0% and 7.0% moisture were compared, the differences did not entirely disappear even after 3½ hours immersion.

By lifting the layer of wet dough off the slick immediately after dipping in the usual way, it was determined that the dough over the low-moisture flour contained quite as much water as that peeled off the high-moisture portion (See Table I). On drying under similar conditions the two doughs gave up their moisture at the same rate. Consequently the color differences cannot be attributed to differences in the amount of water absorbed with resulting variations in the structure of the doughs.

The true explanation for the difference in slick color between high-moisture and low-moisture portions of the same flour was found to lie in the thickness of the film of dough formed on dipping. Each of a few widely different flours was divided into two parts and both portions were exposed at room temperatures, one to dry air and the other to moist air. The two parts of each sample were then slicked on large plates and after partially drying in the oven, measured areas of the crust were cut out, the adherent flour was brushed and scraped off, and the weight of the moisture-free crust was determined.

The following results were obtained:

TABLE I

Flour	Ash	Protein	Moisture	Moisture in dough film immediately after dipping	Wt. of moisture-free crust per sq. in. of surface
	%	%	%	%	gm.
Spring wheat patent	0.40	11.0	15.2	61.4	0.59
" " "			6.2	62.1	0.38
Spring wheat clear	1.25	14.2	14.9	61.0	0.74
" " "			5.9	62.3	0.47
5th break flour	1.38	19.6	14.7	60.6	1.00
" " "			6.1	62.0	0.57
Ontario winter	0.505	9.5	15.6	55.1	0.55
" "			6.2	55.2	0.35
Pacific coast pastry	0.42	7.1	15.2	50.7	0.44
" " "			6.5	51.8	0.30

The dough over the high-moisture flour, because it contains more material, dries out more slowly than that over the low-moisture sample placed beside it. At the time of judging for Pekar color, the former not only contains more dry material per unit area, but also a greater percentage of water. It is, therefore, definitely thicker and more

opaque than that lying over the low-moisture flour. In all the above examples the difference in thickness was quite apparent to the eye. That even small differences in the thickness of the crust have a considerable effect upon the apparent color is easily seen from a few obvious, though crude, experiments, such as reducing the thickness by scraping, or increasing it by adding thin films of dough. If a dough is rolled out into a very thin film, and single, double, and treble thicknesses of this film are placed beside each other over a dipped slick of high grade flour, the progressive darkening in color with thickness of film until opacity is reached may be readily seen.

Portions of the same flour, but of widely different moisture content, were made into doughs so that both doughs contained the same proportions of water and moisture-free flour. The two doughs were rolled out side by side, making adjacent portions of both of the same thickness. When partially or completely dried no line of demarcation between the two doughs could be seen, tho when tested by the Pekar method the high-moisture portion appeared to be a decidedly poorer grade of flour than the drier part.

The good slick color of high grade flours, as compared with clears, and of soft flours as compared with hard, is apparently partly due to the thickness of the dough formed when these flours are dipped in water.

Conclusions

The thickness of the layer of dough formed when making the Pekar test, and consequently the color of a flour as judged by the Pekar method, are influenced to a marked degree by the moisture content of the flour.

Before flours are compared by the Pekar method, they should be brought to approximately the same moisture content by exposure to air of the same relative humidity until equilibrium is reached.

POINTS FOR CONSIDERATION IN BAKING TESTS

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(Read at the convention, May, 1929.)

Chemists are familiar with the difficulty encountered in the standardization of analytical methods for collaborative work. In such analytical methods the conditions pertaining to time, temperature, reagents, and procedure for conducting the test can be very definitely stated. The reactions involved are usually of a known chemical or physical nature. Many years of research have been required to bring these analytical methods to their present degree of precision.

Approximately a year has elapsed since our society tentatively adopted specifications for the Standard Experimental Baking Test. This is therefore our first operative year under such specifications. In analytical methods the personal element is, to a great extent, minimized, while in the baking test such is not the case. The variables are many and methods of measurement few. Yet most cereal chemists consider the baking test as the final criterion of a flour. Because the baking test lacks many of the more refined measurements of an analytical procedure, some chemists have become lax in controlling conditions well within their power.

It is the distinct purpose of this paper to present suggestions in the hope that such may be of value in the future development of standard baking tests. The material for this paper has been obtained from my experimental baking and the result of this year's collaborative work. There has been a tendency on the part of some members of our association to attribute the widely varying results to lack of ability and unfamiliarity of the collaborators with the standard experimental test. Therefore the slides and data presented in this paper are based solely on the work of men of excellent scientific standing in our society. Practically all of the collaborators whose data are here presented are members or past members of the baking committee. Most of them have had several years experience with essentially the same baking method before it was adopted as the standard procedure. I think you will agree that among these experienced collaborators, widely varying results have been obtained. As scientists, let us not dismiss lightly the lack of agreement in our results. A tentative method has been adopted, but to perfect it will require several years of intensive research on factors causing these variations.

Using a portion of some of the flour sent out for the collaborative work and employing the basic procedure of the Standard Experimental Baking Test, many doughs were made. Prior to the time the doughs were ready to pan no variables were introduced.

Quoting from the instructions relative to panning, "Place dough on table or molding board, and pound vigorously with heel of the hand until the dough is flat and circular, as in 1." The instructions are definite, yet the energy exerted will vary according to the individual conception of the term "vigorous."

Series A of these doughs was subjected to an exceedingly vigorous pounding; Series B was pounded less vigorously; Series C was very lightly pounded.

To record some numerical measure of the treatment thus accorded each series, the density after pounding and molding was determined. In each series 30 replicate doughs were treated as nearly alike as possible. Ten of these doughs were used for density determinations. This was accomplished by immersing a known weight of dough in kerosene. The other 20 doughs were proofed and baked.

Average values for each series have been reported. To convey an idea of the type of loaf obtained in each series, reference will be made to the photographs reproduced in Cereal Chemistry Vol. V, No. 4, pages 292-294, by M. J. Blish. For those in attendance at the convention, lantern slides have been prepared to give a more definite idea of the results.

Series A, with the drastic pounding, had an average density of 1.04 and gave a loaf volume of 400 cc. Every dough baked in Series A gave little oven spring and produced a small loaf having no break, namely, Type J.

Doughs in Series B had an average density of 0.90 and those baked gave loaves with an average volume of 520 cc. All doughs in Series B had an excellent oven spring, resulting in loaves of large volume, with a well defined break, resembling Type G.

The doughs in Series C had an average density of 0.85 and those baked had an average loaf volume of only 380 cc. The oven spring was practically negligible. The loaves produced were of small volume with a ragged top resembling no particular type. Type K more closely resembled these loaves than any of the other standard types. Series C is of minor importance, but Series A and B are exceedingly interesting. Series B produced larger loaves than Series A for two distinct reasons: (1) By density calculations it is found that the doughs in Series B immediately after panning had from 18 to 20 cc. more gas

than those in Series A. As the proof is to a constant time rather than volume, the 20 cc. additional gas in Series B may be considered the equivalent of lengthening the proof period. (2) The doughs of Series B were not subjected to as severe manual treatment as those in Series A.

TABLE I

COLLABORATIVE RESULTS ON SAMPLES 1, 2, AND 3

Collaborator No.	Collaborative Sample No.	Baking test used	Gas volume produced by yeast	Loaf volume	External type	Crust color
			cc.	cc.		
15	1	Basic	727	394.1	J	Grayish brown, very mottled
15	2	Basic	719	436.8	J	do
15	2	Supplement C	...	398.0	J	do
15	3	Basic	705	377.0	J	do
15	3	Supplement B	...	371.4	J	do
1	1	Basic	...	457.0	J	Straw
1	2	Basic	...	540.0	J	do
1	2	Supplement C	...	531.0	J	do
1	3	Basic	615	440.0	J	do
1	3	Supplement B	...	415.0	J	do
28	1	Basic	667	.....	J	Top brown, sides of loaf white
28	2	Basic	652	506.0	J	do
28	2	Supplement C	...	493.0	J, tendency towards F.	do
28	3	Basic	575	540.0	J	do
28	3	Supplement B	...	495.0	J	do
12	1	Basic	...	540.0	Break on both sides of loaf	Black, burnt
12	2	Basic	575	535.0	Sl. break on both sides of loaf	do
12	2	Supplement C	...	517.5	Break on both sides of loaf	do
29	1	Basic	410	528.0	H-G	Brown
29	2	Basic	465	519.0	G-F	do
29	2	Supplement C	...	555.6	G-F	do
29	3	Basic	450	458.0	G-F	do
29	3	Supplement B	...	460.6	G-F	do

Often flours are baked that even tho given a severe treatment, as in Series A, produce bread of relatively much larger volume and exhibit a smaller differential than those between Series A and B. This is possibly due to the different capacities of doughs of various flours in resisting mechanical or manual treatment during the panning process. In industrial work this factor is of vital importance. The degree of manual treatment given doughs produced according to the basic procedure, from a given flour, has a great influence on the type of loaf obtained from that flour. Density measurements may aid collaborators in exercising more nearly the same physical treatment in panning.

An attempt will be made to show how this personal factor in panning has had a determining influence upon the results obtained in the collaborative work.

Table I gives the results obtained by some of the collaborators This table has been shortened, owing to the limited time for presenting this paper. In column 5 of this table is recorded the average loaf volume. In several instances it was found that differences as great as 125 cc. were reported by collaborators using their own methods of measurement. I made a few measurements on the loaves sent to me with a well calibrated volume box and a dependable method.

When thus measured, the volume differences were not more than 20 cc. These measurements were taken after shipment, and there was a slight shrinkage in loaf volume. It is urged that the calibration of volume boxes and measurement methods should receive as much care as volumetric flasks used in chemical analysis. In column 6 of Table I is found the external type the loaf nearest approaches. This, together with the lantern slides presented, will greatly assist us in drawing conclusions.

All collaborators baked Sample 1 by the basic procedure; Sample 2, first by the basic procedure and second by supplement C, with the addition of 1 mg. of potassium bromate; and Sample 3, first by the basic procedure and second by supplement B, with 4 hours fermentation. On examining the slides made from the loaves of collaborator No. 15, we find that all loaves obtained were almost identical in appearance. So distinctive were the shape and the peculiar markings on these loaves that they could be readily picked from those of the other collaborators. Our conclusion is that regardless of the flour or the supplement employed, this collaborator has a distinctive "panning personality." His "panning personality" is so much in evidence that to a large measure it covers up the differences that exist in the flours. Without solicitation on my part I received a communication from this collaborator from which the following quotation is taken. "I believe variation in volume is probably due largely to molding the loaf. Our tendency, in this laboratory, I believe, is to make the molding too severe. In these tests Mr. —— has tried to carry out directions as explicitly as possible but I believe that there is probably a lack of uniformity in molding." This confirms our conclusion on the effect of "panning personality."

Collaborator No. 1 likewise has such a distinctive "panning personality" that on the 3 collaborative samples, loaves of the same shape and characteristics were obtained, as is readily observed from the slide.

These loaves had such a distinctive character that they could readily be selected from those of all other collaborators. Here again the "panning personality" has greatly overshadowed the differences in the flours.

Collaborator No. 28 secured from all 3 samples, loaves that resembled each other. The slides show their similarity in shape and peculiar markings. Again, as in both cases previously cited, the loaves of this collaborator could be easily selected from those of the other collaborators by their distinctive appearance. To quote from a communication from him: "Our new baker molds tighter than our previous baker, resulting in an entirely different type loaf." Here again is further confirmation of our conclusions on the effect of "panning personality."

On Samples 1 and 2, collaborator No. 12 secured a very interesting type of loaf. From these slides you will see that there is a slight break on the side of the loaf. The break was on both sides of the loaf, making it appear much like a commercial type loaf. None of the standard photographs resembled this loaf. The fact that a specific type of loaf was secured greatly different in its characteristics from any of the standard types impresses upon us the influence of the personal factor in the panning of doughs.

Thus far, the results of the collaborators that have been cited are no doubt caused from molding similar to that accorded the doughs in Series A.

For the sake of brevity, the slides and data of only one collaborator who gave a molding treatment closely approaching Series B will be cited, namely, collaborator No. 29. Loaves baked from the 3 flours have a decided oven spring, resulting in a prominent break, giving loaves of large volume. Loaves from Samples 1, 2, and 3 had no distinctive appearance to enable one to select them from loaves produced by other collaborators. Indeed, this collaborator is lacking in "panning personality," the absence of which allows the differences in the flours to appear.

Harrel (1926) called attention to the undesirability of pan greasing in baking. Greasing resulted in a considerable decrease in volume. The slide presented in 1926 is again shown. Herman and Hart (1927) pointed out that the same influence was present when doughs were baked in pans of essentially the same dimensions required by the Standard Experimental Baking Test. L. D. Whiting and I have recently conducted similar greasing experiments, employing the standard pans. Certain flours produce loaves having an average of 50 cc.

smaller volume when the pan is greased. Other flours showed little, if any, difference. It is believed the cause of the volume difference lies in the fact that in a greased pan adhesion of the dough to the sides of the pan is greatly lessened, resulting in approximately the total weight of the dough resting on a cross-section represented by the bottom of the pan. In pans that are not greased, part of this weight is supported by the adhesion of the dough to the sides of the pan. Some doughs are so weakened by fermentation that they are unable to stand the increasing weight due to pan greasing, smaller loaves resulting.

In addition to decreasing volume, pan greasing has a very definite influence on loaf appearance. In Figure 1 the left half is a loaf baked in an ungreased pan; the right half is a duplicate dough baked in a

Fig. 1. Influence of Pan Greasing on Appearance of Loaf

greased pan. When greasing was dispensed with, the dough adhered to the sides of the pan causing the crater-like holes, as shown. In the greased pan there was little adhesion and the gas was not entrapped between the dough and the sides of the pan; a very smooth sided loaf resulted.

The answers to the questionnaire sent out this year indicated that about 30% are not greasing, 40% are greasing lightly, and 30% are greasing heavily. Until some definite practice can be attained in so simple a matter, results in baking will vary widely.

Table II contains data obtained from one of the collaborative samples.

TABLE II

A COMPARISON OF THE GAS PRODUCTION AND CRUST COLOR OF SAMPLE 3

Collaborator No.	Position in photograph reading from left to right	Gas production in 105 minutes, cc.	Crust color of baked loaf
12	1	575	Badly burnt
19	2	620	Very brown
29	3	450	Brown
14	4	651	Pale
4	5	753	White

Fig. 2. Variation in Crust Color

Column 3 gives the gas displacement of the doughs up to the first punch, 105 minutes. The gas was collected over kerosene by employing a standard gas collection apparatus. In column 4 of this table is a description of the crust color. Figure 2 gives a relative idea of the enormous variation in crust color. Reading from left to right, the loaf of collaborator No. 29, with a gas production of 575 cc., has a burnt crust, so black that the presence of the slice can be told only by the white spot which appears at the top and is part of the break of the loaf. This loaf was badly burnt. The crust was about 4 mm. thick. On the extreme right, we find a crust that is not only pale but actually white. This slice was taken from a loaf baked by collaborator No. 4. The gas production was 753 cc. Between these two wide extremes are various gradations of crust color. It is inter-

esting to note that the third, fourth, and fifth slices in this photograph have crust color that successively becomes lighter, while the gas production, as found by the collaborators, is increasing. The badly burnt crust on the extreme left and the white crust on the extreme right strongly point to the possibility of great variation in oven temperatures.

Figure 3 furnishes sufficient evidence to draw the conclusion that temperatures have varied. Reading from left to right, slice one was from a loaf with 4 hours fermentation, supplement B; slice 2 was made from the same flour and by the same collaborator, No. 28, but by the basic procedure. The slice from the 4-hour loaf has a much browner crust that that from the 3-hour loaf. Granting correct weighing and

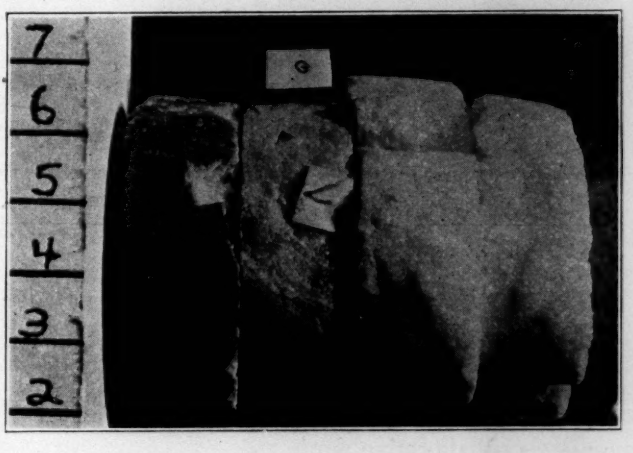

Fig. 3. Comparison of Crust Color on 3- and 4-Hour Fermentation Periods

measurements, such a difference can be explained only by considerable variation in the baking temperatures. Slices 3 and 4 were made from the same flour but by collaborator No. 4. They have fermentation periods of 4 and 3 hours respectively. The crust is white and no difference is observed between the 4- and 3-hour fermentation periods. This strongly suggests that for some reason this collaborator's doughs are exceedingly old even with 3 hours fermentation, or that the oven had not enough heat to brown them.

Figure 4 limits the discussion to oven temperatures. This loaf was baked by collaborator No. 9, using the same flour. The top view, at the left, has a burnt crust. The side view, at the right, is so white and produced a photographic negative so dense that the markings on the side cannot be observed without a greater exposure in printing. When given the proper exposure for the side view, the top view is so black that it disappears in the picture. The artificial source of

illumination in these photographs was 20 feet from the object photographed. As the objects were placed side by side, color differences shown by the slide and photograph are real, not due to lighting effects. This is truly a case of a badly burnt top and an underbaked side. Ovens need to be more thoroly standardized.

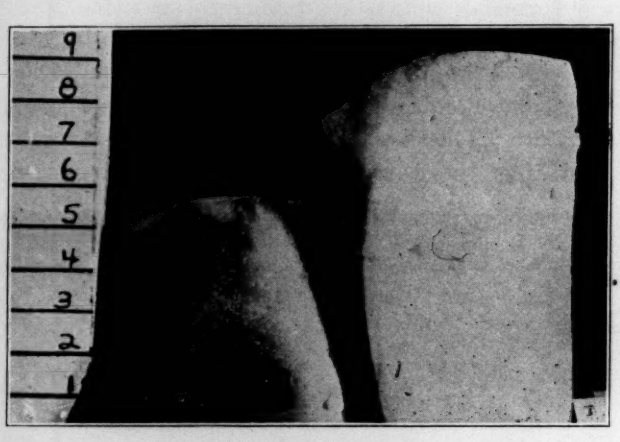

Fig. 4. Loaf With Burnt Top and Underbaked Side

Summary

1. Results obtained by use of the Standard Experimental Baking Test are greatly affected by hand panning.

2. "Panning personality" is one factor that caused wide variations in this year's collaborative work.

3. Density determinations might help collaborators to pan doughs more nearly alike.

4. Molding machines have solved this problem in some commercial laboratories and they will probably have to be employed ultimately in the Standard Experimental Baking Test.

5. A uniform practice in regard to pan greasing should be adopted by collaborators.

6. Enormous variations have been observed in this year's collaborative work. Some of these variations may, in part, be due to the type and biological condition of the yeast.

7. Variations in crust color shown by badly burnt loaves are probably due to lack of proper oven temperature control.

8. A browner crust on a longer fermentation period eliminates the yeast factor and indicates improper temperature control.

9. Ovens must be more thoroly tested and standardized in order to prevent burnt tops with underbaked sides.

10. A tentative method has been adopted. To perfect it or any other method will require further painstaking work.

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1926 Some variable factors of bread production. Cereal Chem 3:1-18.
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REPORT OF COMMITTEE ON METHODS OF ANALYSIS

M. J. BLISH, Chairman

Department of Agricultural Chemistry, University of Nebraska, Lincoln

(Read at the Convention May, 1929)

The methods committee of the last year consisted of F. A. Collatz, A. E. Treloar, L. H. Bailey, and M. J. Blish, chairman. These represent an entire change of personnel from a group that had functioned efficiently for three consecutive and immediately preceding terms.

It is difficult properly to evaluate the work and accomplishments of the various methods committees that have served the association from time to time. There can be no doubt, however, that such work has materially increased the care and uniformity of procedure in the use of our established methods for moisture, ash, and protein. However, altho recommended specifications for the analysis for each of these constituents have been formally adopted by the association, there are still variations among different laboratories as to details and control of operations. This is especially true of the protein test, which has come to play so prominent a part in commerce with wheat and flour. Nevertheless, there is evidence for the belief that such serious analytical discrepancies as occasionally occur are attributable to careless or faulty technic, rather than to any inherent inaccuracy or unreliability of methods.

The large amount of collaborative testing of "check" samples, as carried on through recent years by both our association methods committees and our local sections, has to a noticeable degree elevated the general level of accuracy in our routine analytical work. Monthly collaborative testing has proved to be a safeguard that amply justifies the existence of a local section even if that section indulges in no other activity. Individuals whose results are consistently out of line may thus have their attention drawn to their systematic errors. The rectification of such is not only to their personal advantage, but adds to the prestige of the association as a whole.

In the selection and pursuit of a definite line of endeavor for the last year, the methods committee was influenced by two principal considerations. The first may be indicated by quoting from Coleman's (1928) report of last year: "It is believed to be time that an interpretation be given to our value for experimental error." The report further recommended "that this association go on record as interpreting the tolerance to be permitted in making protein determinations." The other consideration has to do with the actual interpretation itself. How is this precise interpretation of experimental error, "tolerance," etc., to be accomplished, and by whom? The matter is not a simple problem in elementary arithmetic. This fact may, perhaps, be appreciated by consulting the paper of Treloar and Harris (1928) on "Criteria of the Validity of Analytical Methods used by Cereal Chemists." In their summary, with reference to collaborative studies that have previously been made by committees on methods of analysis, the authors state "the value of these efforts may be very largely lost unless careful and accurate study is also made of the resultant data by the refined mathematical methods of the biometrician."

In other words, in regard to the validity of our analytical methods, with special reference to their capability of producing comparable or equivalent results in different laboratories, we are in a position similar to that of a large industrial organization, which finds itself in need of having its books audited and a statement prepared by an expert of special training. With due recognition of this, Mr. Treloar was asked to serve on this committee for the year now at a close, with a view of placing the work of the committee on a basis that would allow the maximum utilization of the collaborative work for the establishment of standards of comparison and bases of deduction.

Variations among the collaborative analytical results of the cereal chemists have always been subjected to more or less comment and criticism of all kinds. For obvious reasons the protein test has more recently occupied the center of the stage. In the collaborative testing of "check samples" uncomfortably wide variations in protein are frequently observed. The association officially recognizes a "tolerance" of 0.25%. What does this mean? How is it to be applied? Is it supposed to apply to wheat, or flour, or both? Is it restricted to a *total range* of 0.25% or do we mean *plus or minus* 0.25%, permitting a total range of 0.50%?

Coleman (1928) calls specific attention to the general existing confusion as to what should be expected in the matter of agreement among laboratories. Closer agreement might be expected where all use the same method than where each uses his own procedure. Indeed,

in this connection, Harrel and Lanning (1929) have recently noted that varying the amount of sodium sulfate may appreciably influence results in protein determinations. Nevertheless, an analysis of Coleman's report (1927) shows that among 58 collaborators no better uniformity of protein results was obtained by the association method than when each collaborator used his own method.

Any collaborative data presenting individual results, and indicating maximum, minimum and average values, will throw some light upon the *extent* of variations among different laboratories. As ordinarily encountered, however, these collaborative data have yielded no precise and reliable information as to fundamental causes of variation nor indeed an accurate measure of it. Such data are frequently misinterpreted from the standpoint of the extent to which they really reflect the general accuracy and reliability of cereal chemists as a whole. In examining the data of any large collaborative group it is to be noted, as a rule, that the large majority check very well, but almost inevitably there are two or three individuals whose results may be regarded as extraordinarily discordant. This wide discordance of a small minority is frequently and unjustly applied as an index to the reliability of the entire group, despite the fact that the results of 80 to 90 per cent of the collaborators were in excellent agreement. This sort of misinterpretation, to cite a concrete case, was undoubtedly the basis for recent unfavorable comments by one of our esteemed critics who had profoundly contemplated the monthly check samples report of one of our local sections. Referring to the maximum, minimum, and average values only, he suggested in an open letter to one of the milling journals that instead of the A.A.C.C. our association should be designated as the G.S.A.—Guessing Society of America.

It would doubtless work to our advantage generally if we could adopt more refined, informative, and adequate methods of evaluating our collaborative analytical data. This would not only improve our own efficiency, but would afford less opportunity for misunderstanding and misinterpretation by non-technical members of the flour and grain trade. To be more specific, it might be well for us to familiarize ourselves with the meaning and use of the term "standard deviation" as ascertained by the simple procedure of the biometrician as an absolute measure of variability, and in reporting our collaborative data to calculate and record this figure, which furnishes a far more accurate and informative measure of the variation among a given group of analysts than does the mere statement of average, maximum, and minimum. This value reflects the analytical reliability of the

entire group, rather than tending to emphasize the shortcomings of two or three individuals. It has the advantage of giving proportionately greater weight to large deviations from the mean than would be given by the simple arithmetic "average deviation." If this is too much to expect, then it would at least be permissible to disregard both the highest and the lowest result in evaluating the collaborative analytical data of any fair-sized group. This procedure is habitually practiced by reputable scientific societies, and it is done merely in recognition of the fact that in spite of all precautions, occasional accidents occur and give extreme results.

The committee's efforts during the last year have been largely under the leadership of Mr. Treloar, and have been confined to a critical inquiry into certain aspects of the protein test. Collaborative data of a special type have been gathered for Mr. Treloar, who has subjected them to statistical analysis. The intended purpose of the work has been to establish within and between laboratories, as a preliminary step toward ascertaining more definitely than has heretofore been possible, the fundamental nature and causes of variations in collaborative protein testing. It is believed that the methods of biometry offer a promising means for the satisfactory solution of problems of this type.

The committee extends its thanks to J. Arthur Harris, of the Department of Botany, of the University of Minnesota, who has taken an active interest in this biometrical work and provided very material facilities for it. The committee's thanks are also extended to the personnel of the laboratories that have collaborated in this protein study. As many as 80 protein tests have been made in each of several laboratories at the committee's request. The detailed report of the nature, scope, and outcome of the last year's collaborative work is to be presented by Mr. Treloar.

In a separate investigation, L. H. Bailey has given special consideration to the matter of indicators as used in the protein test, upon which work he will submit a brief report.

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1929 Relation of quantity of sodium sulfate to time of digestion in protein determination. Cereal Chem. **6**:72-78.

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1928 Criteria of the validity of analytical methods used by cereal chemists. Cereal Chem. **5**:333-351.

Recommendations

The recommendations herewith submitted for the possible consideration of future methods committees are general rather than specific. Possibly the time is at hand when the association may appropriately take steps to formulate, more definitely than has been done, a policy or plan of procedure to guide the activities of our future committees on methods of analysis. The announcement of new committees is generally the last official act of our annual meeting. These committees seldom have an opportunity to even meet, much less to learn the wishes of the association as a whole. Opportunity should be provided for at least one meeting of the entire committee before embarking upon a program of work for the coming year.

There is now available a large accumulation of more or less comprehensive surveys and collaborative data indicating the extent to which individual laboratories may be expected to vary from one to another in collaborative tests for moisture, protein, and ash. Future surveys of this type may appropriately be confined to the sending out of monthly check samples by the local sections. Continued prospecting over a wide range of territory is useful and essential. In addition to this sort of work, however, it is very desirable that we dig deep below the surface, but this is admittedly slow and painstaking work which can be done only within a small and narrowly restricted area at any one time. It is the latter activity, perhaps, to which our methods committees should increasingly direct their attention. As an illustration of this type of work the recent paper "Relation of Sodium Sulfate to Time of Digestion in Protein Determination," by Harrel and Lanning (1929) may be cited. The committee has been able to submit preliminary but definitely quantitative standards for the variability of the recommended method. Quantitative standards of this type should serve as a basis of comparison for variations of method such as that mentioned above. The committee feels that among other matters, intensive investigation of factors influencing time of digestion will prove exceedingly profitable. The majority of laboratories digest samples for a considerably shorter period than that recommended by the association. These shorter digestions may be justifiable in many cases and may represent a highly valuable economy of time; in extreme cases, however they may be responsible for an important incompletion of digestion.

In connection with the protein testing of carlots of wheat, the error of sampling, with its consequences, is an item that possibly deserves closer attention than it has received. Cereal chemists are too

frequently held reponsible for analytical discrepancies that may not be due to analytical errors at all, but to the fact that samples are occasionally neither uniform nor representative. Many carlots are so lacking in uniformity that, even with the utmost care in sampling, one cannot be certain that the sample is representative. Instances may be cited of cars resampled 10 or 12 times, each sample showing a sufficiently different protein content to arouse comment or controversy.

Premiums, disputes, demands for price adjustments, etc., are and have been frequently based upon wheat protein variations of 0.1 or 0.2 per cent. These variations are well within the limits of error of determination, even assuming absolute uniformity of samples. Such hair-splitting appears all the more absurd when the likelihood of additional and serious error of sampling is appreciated. It is possible, however, that the chemist himself contributes materially to this absurdity when he insists upon reporting his protein results to the *second* decimal place, thus creating the impression of a higher degree of refinement and reliability than actually exists. If the second figure after the decimal is without significance in commercial protein tests, why should we persist in thus reporting it? The trade is likely to expect accuracy to the second decimal place as long as cereal chemists pretend to furnish it.

Accordingly this committee presents the following recommendation to the association:

1. That since replicated averages of three determinations within a laboratory may vary over a range of as much as 0.2 per cent protein, this committee recommends that the reporting of protein determinations for commercial purposes to a supposed degree of accuracy greater than one tenth of one per cent be officially regarded with disapproval by the association, as it exposes the profession to criticism that may readily be justified.

To encourage endeavors toward the greatest possible consistency of results among and within all laboratories, the committee further recommends:

2. That every laboratory carefully check its equipment periodically by making a large series of determinations at the one time upon a carefully selected typical flour and by comparing the variability so found with the standards submitted in this report or established upon more extensive data in the future.

3. That sectional activities in check testing be continued with a view toward reducing and if possible eliminating such systematic deviations as characterize individual laboratories, and that a periodic inter-

sectional check test be undertaken to ensure that local sections of the association are all adhering to a common standard.

The committee also suggests that brom cresol green be given a trial by members of this association as an indicator in ammonia titrations.

A STATISTICAL STUDY OF COLLABORATIVE PROTEIN DETERMINATIONS

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(Read at the Convention May, 1929)

Introduction

The physical equipment of a modern chemical laboratory devoted to cereal investigations is usually characterized by a high degree of refinement and precision. Balances are accurate, often to one ten-thousandth of a gram. Burettes may be fully relied upon to one-twentieth of a cubic centimeter. Chemicals are of good quality, and theoretically any error due to them may be checked by "blank" determinations. From these considerations alone, one might expect such a determination as that of protein to give replicates agreeing within a total range of 0.05%. Indeed, as an association of cereal chemists we express confidence of this degree of accuracy in our results by reporting protein analyses to one hundredth of one per cent. This procedure is also in accord with the recommendation of the association's book "Methods for the Analysis of Cereals and Cereal Products."

Official endorsement of the desire to secure the highest possible accuracy within the limits of physical equipment is to be commended. One should not, however, allow the ideal of the highest precision to obscure the facts of the discrepancies actually encountered in routine determinations. Theory and the results of practice are frequently discordant because theory fails to take count of all the factors in practice. It is not wise to allow theory to define the limits within which we might be expected to agree on chemical analyses unless it can be shown that the theory is adequate. The methods committee believes that the association would unanimously endorse the contention that we have not as yet a theory of the variation to be expected in the protein determination that is in accordance with the results of practice. Our standards of

variation should be defined from actual results secured within laboratories of high repute.

It has been the major endeavor of the present committee to take the first steps in clarification of the controversial problem of the closeness of agreement that may reasonably be expected between laboratories when reporting analytical determinations upon the same sample. The protein determination was selected as being perhaps the most important single analysis in the modern cereal laboratory. Conflicting opinions concerning the accuracy of the determinations of this constituent in wheat and flour as reported by different laboratories have frequently led to severe criticism of the analysts concerned, chiefly on account of the lack of adequate knowledge of the variation to be expected. Statistical analysis of the reports of previous committees on methods of analysis has revealed that the determination of protein in flour is more refined than that of ash or moisture. Accordingly, the committee this year aimed to determine the extent of variation in analyses for protein in the flour under rigidly specified procedure within highly reliable laboratories and between them, both when grouped in one section and when widely separated geographically, and has endeavored so to arrange the study as to throw light upon such possible causes of this variation as may be due to equipment or personnel.

Source and Nature of Data

Two extensive series of data have been subjected to careful statistical examination. The first series embraced eight collaborators, seven of whom were active members of the northwest section of this association. A high grade baker's patent flour, designated hereafter as flour sample *A*, of approximately 11% protein, was used. The second series, of wider scope, comprised nine collaborators distributed over a large area. Two patent flours were used, one of high protein content (15% approx.) which will be referred to as sample *B;* and the other, of rather low protein content (10% approx.), sample *C*. These three samples were especially chosen to represent a wide range of protein content in bread flours, thus affording opportunity to determine whether the variation might differ from one sample to another or in relation to the amount of that constituent.

Precaution was taken by the committee to insure that, as far as possible, the results secured by the collaborators were strictly comparable for each sample. It has already been shown (Treloar and Harris, 1928) that there is greater variability among analysts in reporting moisture in flour than in reporting protein, hence it was not desirable to leave in the hands of collaborators the adjustment to a constant moisture

basis. Accordingly the flours were carefully air dried and sent out in air-tight containers, from which the collaborators were asked to make their weighings immediately upon opening, and all at one time, and to report their protein determinations as found. The recommended procedure as given in the Book of Methods (pp. 16-20) was followed, with some special precautions. The periods of digestion asked for were:

Electric heaters—400-450 watts— 60 min.
450-550 watts— 50 min.
550 watts, or over—45 min.

Gas heaters—left to judgment of comparable time by analysts using them.

It was believed that these periods would give complete digestion in all cases. *Fresh* methyl red indicator was stipulated, but the definite shade of color for the endpoint was left to the custom or preference of the analyst. Special bottles were supplied to the collaborators for forwarding samples of their acid and alkali solutions to Dr. Blish, who checked them against critically prepared solutions, using standard certified burettes.

The committee made an unusual call upon its collaborators in asking for 20 determinations upon each sample, together with 20 blank determinations on a specially distributed sample of highly purified sucrose to be digested upon the same heaters. The object of this was two-fold. First, it was thought thus to provide reliable measures of the variability encountered within each laboratory, which would serve as valid bases of comparison. Second, it was proposed accurately to determine how much of the variability of the protein test was represented in the blank and whether there was any common effect upon both total protein and blank associated with the digestion units.

The first series of data, presented as Table I, were submitted to mathematical analysis and the conclusions provided the basis of judgment for the subsequent organization of the second series of collaborative determinations, the results of which are presented in Tables II and III.

TABLE I

FLOUR SAMPLE *A*. ANALYTICAL RESULTS FROM COLLABORATORS FOR TOTAL PROTEIN AND PROTEIN IN THE BLANK

Digestion unit	Collaborator 1		Collaborator 2		Collaborator 3		Collaborator 4	
	Total protein	Blank	Total protein	Blank	Total protein	Blank	Total protein	Blank
1	11.71	0.18	11.65	0.05	11.77	0.10	11.94	0.30
2	11.80	0.16	11.70	0.00	11.72	0.10	11.94	0.28
3	11.72	0.17	11.65	0.05	11.87	0.10	11.83	0.28
4	11.77	0.17	11.60	0.05	11.87	0.10	11.90	0.27
5	11.78	0.17	11.70	0.00	11.82	0.10	11.91	0.29
6	11.78	0.16	11.65	0.05	11.87	0.10	11.96	0.26
7	11.72	0.18	11.70	0.05	11.77	0.10	11.93	0.27
8	11.73	0.18	11.70	0.00	11.82	0.15	11.96	0.29
9	11.76	0.16	11.60	0.00	11.77	0.10	11.96	0.31
10	11.77	0.16	11.55	0.00	11.77	0.15	11.96	0.29
11	11.71	0.16	11.65	0.05	11.77	0.15	11.93	0.28
12	11.80	0.18	11.55	0.05	11.87	0.15	11.99	0.27
13	11.76	0.18	11.60	0.10	11.72	0.10	11.96	0.27
14	11.79	0.17	11.70	0.10	11.72	0.10	11.98	0.26
15	11.78	0.17	11.65	0.10	11.87	0.10	12.00	0.25
16	11.78	0.17	11.65	0.00	11.87	0.10	11.98	0.26
17	11.75	0.18	11.65	0.05	11.82	0.10	11.92	0.29
18	11.75	0.16	11.60	0.05	11.72	0.10	11.97	0.29
19	11.79	0.17	11.65	0.05	11.77	0.10	11.93	0.25
20	11.77	0.17	11.70	0.15	11.77	0.10	12.00	0.27

Digestion unit	Collaborator 5		Collaborator 6		Collaborator 7		Collaborator 8	
	Total protein	Blank	Total protein	Blank	Total protein	Blank	Total protein	Blank
1	11.65	0.10	11.75	0.20	11.87	0.15	11.73	
2	11.65	0.05	11.75	0.20	11.95	0.20	11.89	
3	11.65	0.05	11.75	0.20	11.84	0.20	11.89	
4	11.60	0.10	11.75	0.20	11.90	0.20	11.81	
5	11.60	0.05	11.75	0.15	11.90	0.20	11.81	
6	11.60	0.05	11.75	0.20	11.90	0.21	11.81	
7	11.55	0.05	11.80	0.15	11.89	0.21	11.81	
8	11.65	0.05	11.75	0.15	11.80	0.22	11.81	
9	11.65	0.05	11.80	0.15	11.80	0.15	11.81	
10	11.65	0.05	11.75	0.15	11.80	0.20	11.81	
11	11.60	0.05	11.75	0.20	11.89	0.20	11.81	
12	11.65	0.10	11.85	0.20	11.80	0.20	11.81	
13	11.70	0.10	11.80	0.20	11.90	0.21	11.81	
14	11.65	0.05	11.70	0.15	11.90	0.22	11.81	
15	11.60	0.05	11.75	0.25	11.83	0.20	11.81	
16	11.65	0.05	11.85	0.20	11.88	0.20	11.81	
17	11.65	0.10	11.75	0.15	11.91	0.25	11.81	
18	11.65	0.05	11.65	0.15	11.92	0.20	11.81	
19	11.70	0.10	11.80	0.20	11.90	0.20	11.81	
20	11.65	0.05	11.80	0.20	11.80	0.20	11.81	

TABLE II

FLOUR SAMPLE *B*. ANALYTICAL RESULTS OF COLLABORATORS FOR TOTAL PROTEIN AND PROTEIN IN THE BLANK

	Collaborator									
	2		9		10		11		12	
Digestion unit	Total protein	Blank	Total protein	Blank	Total protein	Blank	Total protein	Blank	Total protein	Blank
1	15.06	0.10	14.88	0.04	15.25	0.17	15.24	0.24	14.96	0.05
2	14.96	0.08	14.84	0.04	15.25	0.14	15.08	0.28	14.91	0.05
3	15.01	0.10	14.84	0.00	15.22	0.14	15.12	0.24	14.96	0.10
4	14.91	0.10	14.84	0.04	15.36	0.17	15.16	0.28	14.86	0.05
5	15.01	0.10	14.88	0.04	15.25	0.14	15.24	0.24	14.91	0.10
6	14.96	0.13	14.80	0.04	15.22	0.14	15.08	0.24	14.96	0.10
7	15.01	0.15	14.96	0.04	15.19	0.11	15.12	0.28	14.96	0.05
8	15.01	0.15	14.84	0.04	15.33	0.14	15.24	0.28	14.86	0.10
9	15.01	0.10	14.96	0.04	15.25	0.11	15.16	0.24	14.91	0.05
10	15.01	0.08	14.96	0.04	15.19	0.09	15.24	0.28	14.96	0.05
11	15.01	0.10	14.84	0.04	15.25	0.14	15.24	0.24	14.96	0.10
12	15.06	0.15	14.96	0.08	15.25	0.14	15.20	0.24	14.96	0.05
13	15.01	0.10	14.80	0.08	15.25	0.17	15.24	0.24	14.86	0.05
14	14.91	0.10	14.84	0.08	15.30	0.17	15.20	0.24	14.96	0.10
15	14.96	0.10	14.92	0.04	15.25	0.14	15.20	0.24	14.96	0.05
16	15.06	0.10	14.92	0.00	15.28	0.14	15.24	0.20	14.91	0.10
17	14.96	0.10	14.88	0.04	15.30	0.17	15.24	0.24	14.96	0.10
18	15.01	0.12	14.84	0.08	15.36	0.11	15.16	0.24	14.96	0.05
19	15.06	0.10	14.88	0.04	15.25	0.14	15.16	0.24	14.91	0.05
20	15.01	0.10	14.84	0.00	15.28	0.11	15.12	0.24	14.96	0.05

	Collaborator							
	13		14		15		16	
Digestion unit	Total protein	Blank	Total protein	Blank	Total protein	Blank	Total protein	Blank
1	15.51	0.32	15.07	0.15	15.19	0.11	15.23	0.28
2	.....	0.32	15.07	0.11	15.22	0.10	15.33	0.39
3	15.11	0.32	15.11	0.11	15.30	0.11	15.23	0.30
4	15.11	0.32	15.03	0.19	15.28	0.11	15.29	0.28
5	15.11	0.32	15.11	0.11	15.22	0.05	15.21	0.26
6	15.11	0.32	15.07	0.11	15.22	0.09	15.10	0.15
7	15.11	0.32	15.07	0.11	15.28	0.10	15.28	0.20
8	15.11	0.32	15.07	0.11	15.19	0.09	15.26	0.28
9	15.11	0.32	14.99	0.11	15.19	0.09	15.24	0.20
10	15.11	0.32	15.07	0.11	15.22	0.11	15.27	0.29
11	15.19	0.32	15.07	0.19	15.28	0.11	15.24	0.25
12	15.27	0.32	15.03	0.15	15.22	0.09	15.28	0.24
13	15.27	0.32	15.11	0.15	15.25	0.10	15.28	0.25
14	15.19	0.32	15.11	0.11	15.16	0.09	15.31	0.20
15	15.31	0.32	15.15	0.11	15.28	0.11	15.23	0.20
16	15.51	0.32	15.15	0.11	15.33	0.11	15.24	0.20
17	15.11	0.32	15.11	0.11	15.22	0.09	15.14	0.20
18	15.31	0.32	15.03	0.11	15.30	0.06	15.26	0.20
19	15.23	0.32	15.07	0.11	15.28	0.11	15.28	0.30
20	15.31	0.32	15.07	0.11	15.22	0.11	15.18	0.25

TABLE III

FLOUR SAMPLE *C*. INDIVIDUAL ANALYTICAL RESULTS OF COLLABORATORS FOR TOTAL PROTEIN AND PROTEIN IN THE BLANK

	Collaborator									
	2		9		10		11		12	
Digestion unit	Total protein	Blank	Total protein	Blank	Total protein	Blank	Total protein	Blank	Total protein	Blank
1	10.01	0.07	9.90	0.00	10.15	0.14	10.21	0.16	9.83	0.05
2	10.01	0.10	9.90	0.04	10.15	0.11	10.05	0.24	9.83	0.10
3	10.11	0.10	9.94	0.00	10.15	0.14	10.09	0.20	9.83	0.05
4	10.06	0.12	9.90	0.00	10.17	0.14	10.13	0.24	9.78	0.10
5	10.06	0.10	9.90	0.00	10.15	0.14	10.17	0.20	9.83	0.05
6	10.06	0.08	9.94	0.00	10.15	0.14	10.01	0.24	9.83	0.05
7	10.06	0.10	9.90	0.04	10.15	0.14	10.09	0.24	9.78	0.05
8	10.11	0.08	9.86	0.04	10.15	0.14	10.05	0.20	9.83	0.10
9	10.06	0.12	9.94	0.00	10.15	0.14	10.05	0.24	9.83	0.10
10	10.01	0.10	9.90	0.00	10.12	0.14	10.17	0.24	9.83	0.05
11	10.11	0.12	9.94	0.00	10.15	0.11	10.17	0.20	9.83	0.10
12	10.11	0.12	9.90	0.00	10.17	0.09	10.13	0.24	9.78	0.05
13	10.01	0.08	9.90	0.04	10.17	0.06	10.17	0.24	9.83	0.05
14	10.01	0.08	9.94	0.04	10.15	0.11	10.13	0.20	9.83	0.05
15	9.96	0.10	9.94	0.00	10.15	0.09	10.17	0.24	9.83	0.10
16	9.96	0.10	9.94	0.04	10.15	0.09	10.13	0.24	9.83	0.05
17	10.11	0.08	9.90	0.04	10.15	0.11	10.13	0.24	9.78	0.05
18	10.06	0.10	9.90	0.08	10.20	0.11	10.05	0.24	9.83	0.05
19	10.06	0.10	9.90	0.00	10.17	0.11	10.13	0.20	9.83	0.10
20	10.06	0.10	9.86	0.04	10.12	0.11	10.13	0.24	9.83	0.10

	Collaborator							
	13		14		15		16	
Digestion unit	Total protein	Blank	Total protein	Blank	Total protein	Blank	Total protein	Blank
1	10.16	0.28	10.11	0.15	10.06	0.11	10.07	0.30
2	10.16	0.32	10.11	0.15	10.12	0.11	10.15	0.38
3	10.24	0.28	10.11	0.11	10.15	0.11	10.17	0.28
4	10.32	0.32	10.11	0.15	10.12	0.11	10.15	0.30
5	10.56	0.32	10.11	0.11	10.20	0.11	10.19	0.30
6	10.36	0.32	10.07	0.11	10.17	0.11	10.13	0.27
7	10.12	0.28	10.03	0.11	10.20	0.09	10.17	0.30
8	10.28	0.28	10.07	0.15	10.19	0.14	10.22	0.25
9	10.16	0.28	10.11	0.19	10.17	0.10	10.19	0.26
10	10.24	0.32	10.11	0.15	10.17	0.09	10.17	0.30
11	10.32	0.32	10.07	0.15	10.12	0.09	10.17	0.27
12	10.24	0.28	10.07	0.15	10.20	0.23	10.18	0.28
13	10.32	0.32	10.07	0.15	10.17	0.10	10.20	0.22
14	10.24	0.32	10.07	0.11	10.12	0.07	10.22	0.25
15	10.20	0.32	10.07	0.15	10.15	0.11	10.17	0.22
16	10.20	0.32	10.11	0.15	10.17	0.11	10.12	0.20
17	10.28	0.28	10.07	0.11	10.17	0.11	10.12	0.20
18	10.28	0.32	10.07	0.11	10.13	0.06	10.17	0.24
19	10.16	0.32	10.07	0.15	10.20	0.09	10.18	0.15
20	10.32	0.32	10.07	0.15	10.17	0.11	10.19	0.25

The collaborators who have provided the data for the committee are believed to represent a cross-sectional group of laboratories in which this association might have every confidence. A list of these referred to in each case by a number, the key to which remains with analysts is submitted herewith. In order to remove any possibility of personalities entering into consideration, the collaborators will be the committee.

Series I—Flour Sample *A*.

W. G. Anderson,	Pillsbury Flour Mills, Minneapolis
F. W. Bliss,	F. W. Bliss Chemical Laboratories, Minneapolis (used gas burners)
F. A. Collatz,	General Mills, Minneapolis
S. J. Durrigan,	Cargill Grain Co., Superior
Marjorie A. Howe,	Russell-Miller Milling Co., Minneapolis
M. D. Mize,	Grain Exchange, Omaha
Cleo Near,	Russell-Miller Milling Co., Billings
W. B. Young,	Minn. State Grain Protein Laboratory, Minneapolis

Series II—Flour Samples *B* and *C*.

F. A. Collatz,	General Mills Inc., Minneapolis
R. K. Durham,	Rodney Milling Co., Kansas City
W. L. Heald,	Larabee Milling Co., Kansas City
H. H. Johnson,	Gooch Milling Co., Lincoln
C. B. Kress,	Sperry Flour Co., San Francisco (used gas burners)
C. E. Mangels,	No. Dak. Agr. Expt. Station, Fargo
R. M. Sandstedt,	Nebr. Agr. Expt. Station, Lincoln (used gas burners)
E. F. Tibbling,	General Mills Inc., Kansas City
L. D. Whiting,	Ballard and Ballard Co., Louisville

The collaborators of Series I reported total proteins and blanks, in several instances with a few additional results obtained by using their customary method or by using metallic mercury instead of mercuric oxide as specified in the recommended method. In the second series, strength of solutions and quantities used were reported and all calculations were made uniformly according to these data by the committee. The protein determinations given in Tables II and III are based upon the solution strengths as given by the collaborator, and not upon those determined by the committee. This plan was followed because while

the solution strengths may be carefully checked, it is not possible to subject the collaborators' burettes and pipettes to similar treatment. The nature of their equipment might well make the values submitted by the collaborators more accurate than those calculated from the "check" strengths of solutions. A comparison of the strength of solutions as submitted and the "check" values determined by the committee is given in Table IV. It will be noted that in the majority of cases the differences between reported normalities of solutions and those determined by the committee are small. In one extreme case, however, the discrepancy is such as to account for a difference of as much as 0.19% protein.

TABLE IV

COMPARISON OF SOLUTION STRENGTHS SUBMITTED BY COLLABORATORS WITH THOSE FOUND BY TITRATION AGAINST CRITICAL STANDARD SOLUTIONS

Collaborator	Acid Normality		Alkali Normality	
	Reported	Found	Reported	Found
Series I				
1	0.1253	0.1248	0.1253	0.1248
2	0.1253	0.1256	0.1247	0.1250
3	0.1250	0.1240	0.1250	0.1250
4	0.1253	0.1252	0.1250	0.1250
5	0.1257	0.1256	0.1253	0.1253
6	0.1253	0.1254	0.1253	0.1256
7	0.1253	0.1256	0.1253	0.1244
Series II				
2	0.1254	0.1270	0.1254	0.1256
9	0.2000	0.2000	0.1000	0.1000
10	0.0714	0.0711	0.0714	0.0707
11	0.1000	0.0998	0.1000	0.0992
12	0.1250	0.1252	0.1250	0.1244
13	0.1179	0.1185	0.1002	0.1004
14	0.1013	0.1022	0.1019	0.1020
15	0.0714	0.0718	0.0714	0.0710
16	0.1250	0.1248	0.1250	0.1238

The mean or average values and standard deviations of the total protein and the blank are readily determinable by well known methods. Thus:

$$\bar{x} = \frac{\Sigma x}{N} \quad \text{.....(i)}$$

$$\sigma_x = \sqrt{\frac{\Sigma(\bar{x})^2}{N} - (\bar{x})^2} \quad \text{.....(ii)}$$

where $\bar{x}$ is the mean value of x, σ_x the standard deviation of x, and Σ indicates summation of the designated values of the variable.

It is desired to ascertain the mean value and variability due to the protein in the flour alone. One may expect the average value of the

blank to vary from one laboratory to another. This is ascribable to variation in chemicals, to divergence in the judgment of different collaborators as to the correct color of the indicator representing a pure solution of the salt of the acid and base used in titration, and also perhaps to differences in physical equipment. At present it is not possible to segregate these quantitatively, altho it will be shown later that one, at least, of these factors must be subject to progressive change during a series of 20 determinations as made by the analyst. It is well to bear in mind that if our chemistry is to have any basis for logical interpretation, there is a true or absolute value for the protein in the flour (which may be symbolized by F') and also one for the "protein" in the blank (which may be designated by f'). Then the determined value, F, is in each case equal to F' plus or minus an error arising from lack of homogeneity of the material or error of determination; i.e. $F = F' \pm E$. Likewise $f = f' \pm e$.

It will at once be seen that the average estimated protein in the flour is given by the difference between the average total protein for that flour and the average associated blank. The variability of the protein in the flour as found in each laboratory may be calculated from the equation for the standard deviation of the difference

$$[\sigma_{(x-y)}]^2 = (\sigma_x)^2 + (\sigma_y)^2 - r_{xy}\ \sigma_x \sigma_y \ldots\ldots\ldots\ldots \text{(iii)}$$

where x and y are the two variables (in this case *total protein* and *protein in the blank),* and r_{xy} designates the coefficient of correlation between them. One might not at first see any reason for correlation between protein and blank determinations made on the same burners, unless (a) there is loss of nitrogen from both and in a measure associated intrinsically with the burner, or (b) there is a progressive error of personal equation affecting the results in twenty consecutive determinations. However, such correlations are herein shown to exist; fuller consideration of their significance will be given in a later section of this discussion.

Table V presents the means and standard deviations of the total protein, protein in the blank, and protein in the flour, together with the averages of all determinations for protein in the flour, and average standard deviations. The latter have been derived from the formula giving correct weighting as follows:

$$\bar{\sigma}^2 = [\Sigma\ (n\ \sigma^2)]/N \ldots\ldots\ldots\ldots\ldots\ldots \text{(iv)}$$

where $\bar{\sigma}$ is the average standard deviation, and n and N are respectively the number of determinations made by each collaborator and by all collaborators.

One would expect the values for the estimated protein in the

flour to be reasonably constant. That is, if standardization of method and equipment be complete, any variation in the average values of protein in a flour sample as analyzed in different laboratories might be expected to be ascribable simply to random sampling. The average value as found by each collaborating laboratory should not differ significantly from the average value of the entire series of determinations. The latter average may be accepted as the most trustworthy measure of the protein in the given sample of flour that the data readily present.

A scrutiny of Table V shows that for flour sample *A* the averages of 20 determinations varied from 11.5725 ± .0050 to 11.6875 ± .0087, covering a range of .1150 per cent of protein; for flour samples *B* and *C* the ranges of the averages are respectively .3115 and .2995 per cent protein. The low probable errors of the individual averages indicate the high dependability of the latter in practically all cases.

TABLE V

AVERAGES AND STANDARD DEVIATIONS OF TOTAL PROTEIN, PROTEIN IN THE BLANK, AND PROTEIN IN THE FLOUR, AS DETERMINED BY EACH ANALYST, WITH AVERAGE OF ALL ANALYSES FOR PROTEIN IN THE FLOUR IN EACH SAMPLE

Collaborator	Total protein		Protein in the blank		Protein in flour	
	Mean	σ	Mean	σ	Mean	σ
			Flour Sample *A*			
1	11.7610 ±0.0043	0.0284	0.1700 ±0.0012	0.0077	11.5910 ±0.0047	0.0311
2	11.6450 ±0.0071	0.0472	0.0475 ±0.0061	0.0402	11.5975 ±0.0088	0.0580
3	11.7975 ±0.0084	0.0558	0.1100 ±0.0030	0.0200	11.6875 ±0.0087	0.0576
4	11.9475 ±0.0058	0.0387	0.2765 ±0.0024	0.0159	11.6710 ±0.0068	0.0453
5	11.6375 ±0.0053	0.0349	0.0650 ±0.0035	0.0229	11.5725 ±0.0050	0.0334
6	11.7650 ±0.0068	0.0450	0.1825 ±0.0043	0.0286	11.5825 ±0.0069	0.0455
7	11.8690 ±0.0071	0.0468	0.2010 ±0.0031	0.0207	11.6680 ±0.0069	0.0455
8	11.8140 ±0.0046	0.0307	....	.....	....	.....
	Average				11.6243 ±0.0026	0.0452
			Flour Sample *B*			
2	15.0000 ±0.0066	0.0436	0.1080 ±0.0031	0.0204	14.8920 ±0.0067	0.0442
9	14.8760 ±0.0078	0.0520	0.0420 ±0.0036	0.0236	14.8340 ±0.0088	0.0583
10	15.2640 ±0.0070	0.0461	0.1390 ±0.0035	0.0230	15.1250 ±0.0066	0.0440
11	15.1840 ±0.0084	0.0557	0.2480 ±0.0031	0.0204	14.9360 ±0.0096	0.0638
12	14.9325 ±0.0056	0.0370	0.0700 ±0.0037	0.0245	14.8625 ±0.0065	0.0432
13	15.2153 ±0.0197	0.1273	0.3200 ±.....	.....	14.8953 ±0.0197	0.1273
14	15.0780 ±0.0059	0.0392	0.1240 ±0.0039	0.0262	14.9540 ±0.0079	0.0526
15	15.2425 ±0.0067	0.0447	0.0970 ±0.0025	0.0165	15.1455 ±0.0066	0.0440
16	15.2440 ±0.0081	0.0537	0.2460 ±0.0080	0.0530	14.9980 ±0.0079	0.0522
	Average				14.9606 ±0.0032	0.0635
			Flour Sample *C*			
2	10.0500 ±0.0070	0.0464	0.0975 ±0.0022	0.0148	9.9525 ±0.0069	0.0455
9	9.9100 ±0.0038	0.0249	0.0200 ±0.0036	0.0237	9.8900 ±0.0060	0.0397
10	10.1535 ±0.0025	0.0168	0.1180 ±0.0035	0.0229	10.0355 ±0.0049	0.0322
11	10.1180 ±0.0079	0.0523	0.2240 ±0.0035	0.0233	9.8940 ±0.0097	0.0644
12	9.8200 ±0.0030	0.0200	0.0700 ±0.0037	0.0245	9.7500 ±0.0044	0.0292
13	10.2580 ±0.0145	0.0959	0.3060 ±0.0029	0.0191	9.9520 ±0.0137	0.0909
14	10.0840 ±0.0035	0.0229	0.1380 ±0.0034	0.0223	9.9460 ±0.0039	0.0262
15	10.1575 ±0.0054	0.0356	0.1080 ±0.0049	0.0325	10.0495 ±0.0060	0.0397
16	10.1665 ±0.0053	0.0350	0.2610 ±0.0073	0.0483	9.9055 ±0.0095	0.0629
	Average				9.9306 ±0.0026	0.0518

However, notwithstanding the consistency of the individual analyses within a laboratory (as shown here by the low values of the probable errors), there are wide differences in average results from laboratory to laboratory. To test for differentiation among the average yields of the laboratories, one may employ the criterion given by Pearson (1906) of the significance of the deviation of the mean of a subsample (m) from that of the whole sample (M). In order to apply this criterion, it is well to note that the standard deviation of the whole sample may be derived from the formula

$$N\,\Sigma^2 = S[n\,\sigma^2] + S[n\,(m\text{-}M)^2] \quad \text{(v)}$$

where Pearson's notation is used, S being employed to denote summation and Σ and σ designating the standard deviation of the sample and subsamples respectively. The results are presented as Table VI, in which criteria of the significance of differentiation are given in columns 4 and 5. It will at once be apparent from these that there is a very significant difference between the results presented by these collaborators on the flours submitted for protein analysis. In the majority of cases the odds against occurrence of these deviations due to random sampling are in excess of a million to one. These odds reach their greatest magnitudes with analysts who show the most extreme deviations from the general mean, and thus clearly indicate the individuality of the results. The committee took precautions to be reasonably sure that the flour samples submitted to the analysts were not differentiated in themselves, hence the differences surely arise from the manipulation, equipment, and diverging judgment of the analysts.

As chemists we should determine what minimum value of the difference between the average analytical result of one laboratory and the general average for all laboratories is *important* for each purpose. There may be a wide difference between significance and importance, according to the objects of an investigation. From a chemical standpoint, the differences between the averages presented by collaborators 1, 2, 5, and 6, in flour sample *A* are within the accuracy of equipment and undoubtedly are unimportant, but the difference between collaborators 9 and 10 for flour sample *B*, or 12 and 15 for flour sample *C*, can hardly be so considered.

There can be no question of the careful and painstaking work of the analysts who have collaborated in this study, and it seems undesirable to suggest that any one is in error. Rather, attention must be drawn to the fact that such discrepancies have actually arisen in data specially compiled for a study of variation within and between laboratories when using the same carefully standardized method, and that differences of

equal or greater magnitude may be expected under ordinary conditions and where diverse methods are employed.

TABLE VI

SIGNIFICANCE OF DIFFERENCES BETWEEN THE MEAN YIELD OF ALL ANALYSES FOR PROTEIN IN THE FLOUR (M) AND THOSE OBTAINED BY INDIVIDUAL ANALYSTS (m)

Collaborator	$m-M$	$E_{(m-M)}$	$\frac{m-M}{E_{(m-M)}}$	Approx. odds
(1)	(2)	(3)	(4)	(5)
		Flour Sample *A*		
1	—0.0333	0.0054	— 6.2	>10⁶:1
2	—0.0268	0.0082	— 3.3	70 :1
3	+0.0632	0.0081	+ 7.8	>10⁶:1
4	+0.0467	0.0068	+ 6.9	>10⁶:1
5	—0.0518	0.0055	— 9.4	>10⁶:1
6	—0.0418	0.0068	— 6.2	>10⁶:1
7	+0.0437	0.0068	+ 6.4	>10⁶:1
		Flour Sample *B*		
2	—0.0686	0.0084	— 8.1	>10⁶:1
9	—0.1266	0.0097	—13.1	>10⁶:1
10	+0.1644	0.0080	+20.6	>10⁶:1
11	—0.0246	0.0105	— 2.4	18 :1
12	—0.0981	0.0082	—11.9	>10⁶:1
13	—0.0654	0.0185	— 3.5	108 :1
14	—0.0066	0.0093	— 0.7	2 :1
15	+0.1849	0.0078	+23.6	>10⁶:1
16	+0.0374	0.0093	+ 4.0	280 :1
		Flour Sample *C*		
2	+0.0219	0.0078	+ 2.8	33 :1
9	—0.0406	0.0072	— 5.8	21000:1
10	+0.1049	0.0063	+16.8	>10⁶:1
11	—0.0366	0.0099	— 3.7	160 :1
12	—0.1806	0.0054	—33.4	>10⁶:1
13	+0.0214	0.0131	+ 1.6	6 :1
14	+0.0154	0.0060	+ 2.6	24 :1
15	+0.1189	0.0069	+17.2	>10⁶:1
16	—0.0251	0.0097	— 2.6	24 :1

There are certain advantages in a graphical presentation of the foregoing data. Figures 1, 2, and 3 present the frequency distributions of the determinations of each collaborator for flour samples *A*, *B*, and *C*. The determinations have been classified into groups of a range of 0.05% protein, and each histogram is placed in its correct position above a common scale on the lower horizontal margin. The mean values for each collaborator and the general mean are indicated by the broken vertical lines. The total distributions for the three flours are given in Figure 4, arranged with their respective means forming a common vertical axis. Figures 1 to 3 show at a glance the relative positions of analysts to one another in their determinations of protein. Certain

outstanding features may be remarked upon: (1) The analysts of flour *A* (Fig. 1) show a much greater concordance of results than those of flours *B* and *C* (Figs. 2 and 3.) In this connection it is worthy of note that all but one of the analysts of flour sample *A* are members of the

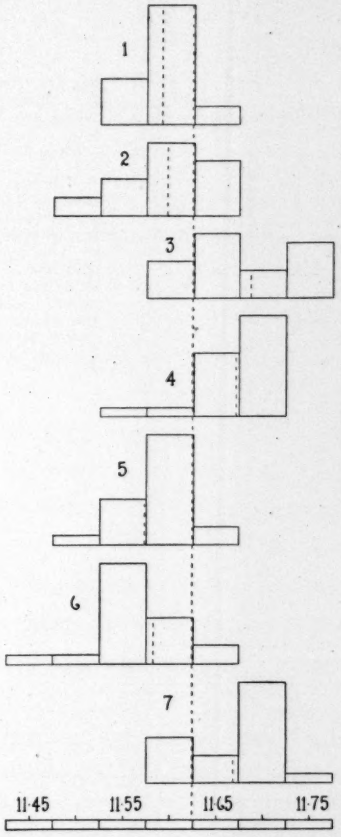

Fig. 1. Flour Sample *A*. Histogramic Distributions of 20 Protein Analyses for Each Collaborator

Frequencies in each class of 0.05% protein presented on the horizontal scale are given by the height of the columns. The mean value for each collaborator, together with the grand average, is given by the broken vertical line in each case.

one local section of this association, and had participated in regular bimonthly testing of the "check" samples of that section. The relation of systematic errors to one another may thus have been materially reduced. (2) Altho no attempt has been made to fit curves to these histograms on account of the small number of determinations in each, it is apparent that marked deviations from a "normal" distribution occur in several instances, and that nearly all distributions are skew or even bimodal. (3) In samples *B* and *C*, representing flours of high and low protein content respectively, the collaborators hold a similar relative position to one another in each case. This is shown

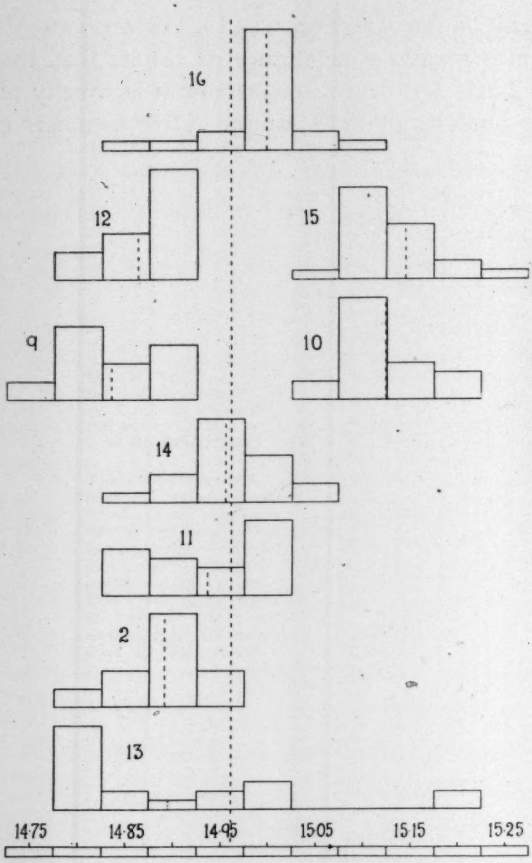

Fig. 2. Flour Sample *B*. Histogramic Distributions of 20 Protein Analyses for Each Collaborator

Frequencies in each class of 0.05% protein presented on the horizontal scale are given by the height of the columns. The mean value for each collaborator, together with the grand average, is given by the broken vertical line in each case.

more strikingly in Figure 5, where the average protein content as determined by each collaborator for flour sample *B* is plotted against his average for flour sample *C*, the diagonal line representing equal deviations from the means of all analysts, given as the horizontal and vertical lines respectively. The distribution of the points shows clearly that analysts who are high in protein determinations on one sample show a marked tendency to be high in the same determination on another sample. The deviations from the general mean are distinctly systematic. This is in agreement with the positive correlation shown by Treloar and Harris (1928) between analyses by two methods of determining ash in flour as obtained by a wide range of analysts. The importance of these systematic deviations or errors is apparent.

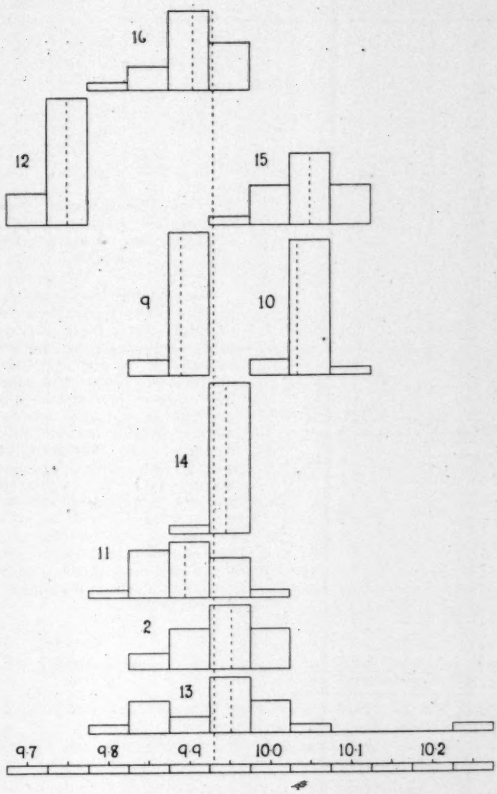

Fig. 3. Flour Sample *C*. Histogramic Distributions of 20 Protein Analyses for Each Collaborator

Frequencies in each class of 0.05% protein presented on the horizontal scale are given by the height of the columns. The mean value for each collaborator, together with the grand average, is given by the broken vertical line in each case.

Concordance of Variability Within Laboratories

While the collaborators show significant and in many cases commercially important differences in the mean yields of the 20 determinations, the same may not be said of the variability of their determinations. It will be seen from Table IV and Figures 1 to 3 that, with one exception, the variation of each analyst is reasonably consistent with that of all other collaborators analyzing the same flour, allowing for effects that might be attributed to random sampling. The average standard deviation of these collaborators forms the most reasonable measure of the variability of protein in the flour samples that may be expected. These weighted averages are given in Table IV and represented graphically ($\bar{\sigma}$) for each flour sample in Figure 4 as the lower horizontal panel in each case. These figures lead to the important con-

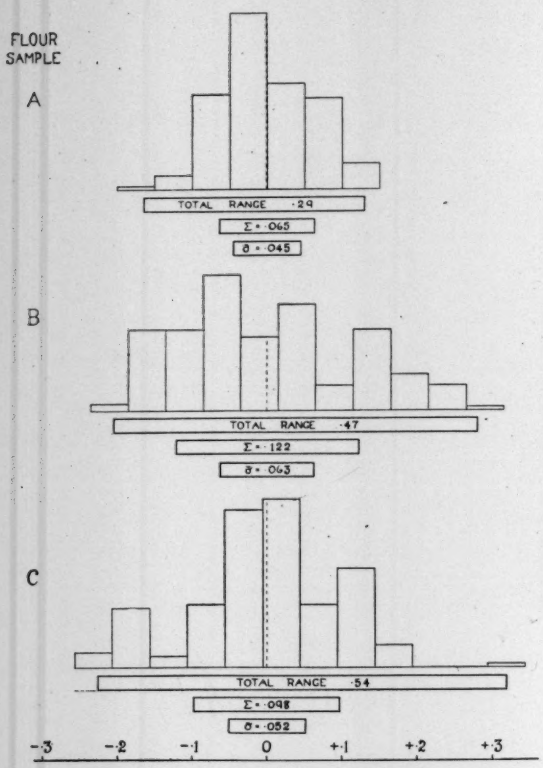

Fig. 4. Histogramic Distributions of All Analyses for Protein in Each Flour Sample

The general averages, given by the broken vertical lines in each case, form the common vertical axis. The lower horizontal scale represents deviations from the mean. The upper horizontal panel represents in each case the entire actual range of all analyses for the flour sample. The lower horizontal panel (σ) for each flour sample represents the average standard deviation of collaborators, while the middle panel (Σ) of each group gives the standard deviation of all protein analyses on the given flour.

clusion that the variability which may be expected in the protein test on a flour sample is not the same for all flours, but varies, perhaps with the type of organic molecular complexes that characterize the proteins in the sample. This variability is not, however, proportional to the amount of protein present, as is shown by the following coefficients of variation, V, where

$$V = \frac{100\,\sigma}{m} \qquad \text{(vi)}$$

Flour sample	Per cent protein	Standard deviation	Coeff. of variation
A	11.62	0.0452	0.39
B	14.96	0.0635	0.42
C	9.93	0.0518	0.52

Thus V ranges from 0.39% for the medium protein flour to 0.52% for the low protein flour, the high protein sample falling between the two.

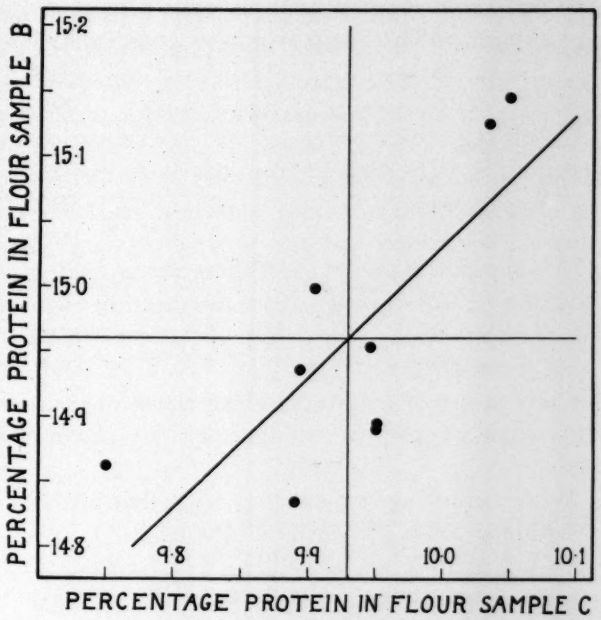

Fig. 5. Average Values for Protein in Flour Samples *B* and *C* as Found by the 9 Collaborators of Series II

This illustrates the systematic deviations of the laboratories concerned. The diagonal line is that of equal deviations from the general averages for the two flour samples, which are shown by the intersecting horizontal and vertical lines.

Standards of Variation Within and Among Laboratories in Reporting Protein in the Flour

What standards of variation for the protein determination may be established from these series, which have been especially compiled for this purpose? It does not appear practicable to set a standard that will hold rigidly for all flours. However, if flour sample *B*, having the largest average standard deviation of the 3 flours, is chosen as a basis for calculation, it seems reasonable to conclude that the variability of the majority of flours milled from the common bread wheats (*Triticum vulgare*) should fall within this range. Upon these premises it is now possible to propose the following standards.

(A) Within any *individual laboratory* exercising careful technic in the determination of protein in flour by means of the method recommended by the American Association of Cereal Chemists:

(i) When reporting one analysis only, that analysis should be within ±.04% protein from the mean in 50% of the cases.
" ±.08% " " " " " 80% " " "
" ±.16% " " " " " 99% " " "

(ii) When reporting average of two determinations, it should be within ±.03% protein from the mean in 50% of the cases.
" ±.06% " " " " " 80% " " "
" ±.12% " " " " " 99% " " "

(iii) When reporting average of three determinations, it should be within ±.02% protein from the mean in 50% of the cases.
" ±.05% " " " " " 80% " " "
" ±.09% " " " " " 99% " " "

(B) Within any *group of laboratories* exercising careful technic in the determination of protein in flour by the above recommended method:

(i) When reporting one analysis only, that analysis should fall within ±.08% protein from the mean in 50% of the cases.
" ±.16% " " " " " 80% " " "
" ±.31% " " " " " 99% " " "

(ii) When reporting the average of two analyses, it should fall within ±.06% protein from the mean in 50% of the cases.
" ±.11% " " " " " 80% " " "
" ±.21% " " " " " 99% " " "

(iii) When reporting the average of three analyses, it should fall within ±.05% protein from the mean in 50% of the cases.
" ±.09% " " " " " 80% " " "
" ±.18% " " " " " 99% " " "

These figures have been obtained by consulting Sheppard's Table of Deviates of the Normal Curve for each Permille of Frequency, published by Galton (1907) and reprinted in Pearson's Tables for Statisticians and Biometricians, in conjunction with the probable error of determination, given by

$$P.E. = .67449\,\sigma \,/\, \sqrt{n} \qquad \text{(vii)}$$

where n is the number of determinations and σ is accepted as .063467 within laboratories and .122237 between laboratories (as derived for flour sample *B*.) The distribution curves derivable from these statistics for variation to be expected both within and between laboratories of high repute in making protein determinations upon the same flour sample are given as Curves I and II in Figure 6. The greater variability between laboratories causes the marked diminished frequency at

the mean as compared with analyses within those laboratories. The vertical parallels mark, in progressive order, the deviations from the means between which 50%, 80%, and 99% of the cases fall. The effect of systematic differences between laboratories is strikingly shown in the much greater width of the comparable area zones in curve II as compared with curve I. For convenient comparison the lines limiting these zones have been projected upon the common scale of deviations from the means inserted between the two frequency curves.

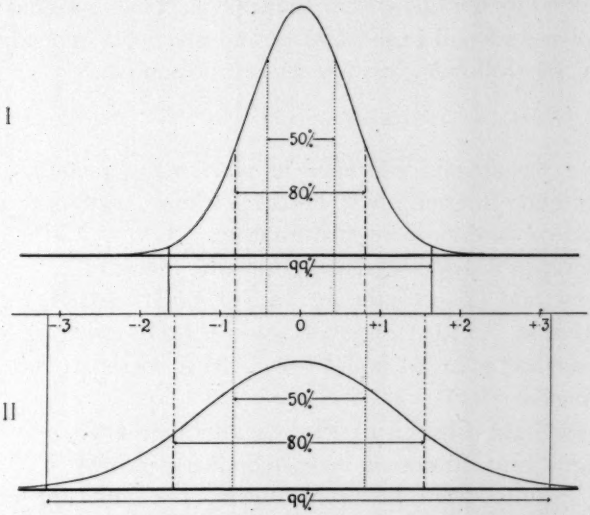

Fig. 6

I. Curve of variation of results of the determination of protein in a uniform flour sample by an analyst using the recommended method.
II. Curve of variation of results of the determination of protein in a uniform flour sample by several analysts working in different laboratories but using the same standardized method.

Now the ultimate aim of standardization among different laboratories should surely be that the variability in the group as a whole should not be greater than the average variability found within the individual laboratories. It is impossible, *prima facie,* to secure less. Actually the variability of all the analyses for this carefully selected group of collaborators is far greater than that found within single laboratories. The standard deviation of all determinations is practically twice as large as that within the laboratories for the same sample. The present series represents the most refined set of collaborative data of such large scope secured by the association. A series of two years ago among 47 analysts reporting average values of at least 2 determinations showed practically the same variability among these averages. In round figures, the present variability among laboratories using the *same* method throughout, is two or more times as great as it

should be. It might be even greater if the different customary methods of analysts were used, but it has been shown (Treloar and Harris, 1928) that the variability when different methods are employed is not significantly different from that when the A.A.C.C. method is used throughout.

Comparisons of the Recommended Protein Test with Other Methods

In several instances in Series I the collaborators presented a few results obtained by methods other than the A.A.C.C. protein test. While the numbers were small in most cases and are not a dependable basis of comparison, the following comments are presented.

Coll. No.

1. (a) No significant difference in yield using metallic mercury.
   (b) Slightly higher yield (+.01%) and lower variability using 25 cc. acid as in regular routine.
2. No significant difference using metallic mercury.
3. Own method (2 gm. sample, Na_2SO_4 only, with H_g) gives 11.5%, that is, A.A.C.C. method gives 0.12% higher yield.
4. Own method gives 11.6025%—A.A.C.C. method gives significantly higher yield.
6. No significant difference using metallic mercury.
7. No significant difference using metallic mercury.

These results seem to indicate that the officially recommended protein test gives a higher yield than those compared with it. Also, the use of mercury in the metallic form gives results equivalent to those obtained with mercuric oxide.

Correlation Studies Within and Between Comparable Series of Protein Determinations

No association between total proteins and blanks made on the same digestion units would theoretically be expected. If such association did exist, however, it would be an important factor in determining the variability attributable to the determination of flour protein alone. It was with the purpose of measuring any unrecognized correlation that paired determinations of proteins and blanks were asked for in this series. These correlation studies are summarized in Table VII. They are surprising and arouse thought as to the origin of these often pronounced associations.

The limited study of this type made possible from the first series of paired determinations (total protein *A* and protein in blank *a*) gave the impression that there was a small but definite positive correlation

TABLE VII

CORRELATION BETWEEN PROTEIN DETERMINATIONS MADE ON THE SAME DIGESTION UNITS

Paired analyses	Series I Collaborator								
	1	2	3	4	5	6	7		
Flour *A* and Blank *a*	−0.2273	+0.1251	+0.0895	−0.2419	+0.3913	+0.3012	+0.2848		
	Series II Collaborator								
	2	9	10	11	12	13	14	15	16
Flour *B* and Flour *C*	+0.0742	+0.0927	+0.4865	+0.6754	−0.0392	−0.2970	−0.0357	+0.0917	+0.3648
Blank *b* and Blank *c*	−0.0331	+0.2151	−0.2684	+0.1008	−0.2500	.....	+0.2887	+0.0262	+0.4449
Flour *B* and Blank *b*	+0.2025	−0.0587	+0.3387	−0.2394	+0.0552	.....	−0.2654	+0.2269	+0.5216
Flour *C* and Blank *c*	+0.2187	−0.3396	−0.2927	−0.3541	+0.1531	+0.3563	+0.3294	+0.3238	−0.1197
Flour *B* and Blank *c*	+0.0775	−0.2600	−0.1202	−0.3202	−0.3587	+0.1526	−0.3482	−0.1789	+0.2473
Flour *C* and Blank *b*	+0.4545	−0.0341	+0.2791	−0.2109	+0.1531	.....	+0.0735	+0.2440	+0.0248
Average (*r*)	+0.1657	−0.0641	+0.0705	−0.0581	−0.0478	+0.0375	+0.0070	+0.1223	+0.2473
Prob. Error $\left(E_r\right)$	±0.0599	±0.0613	±0.0613	±0.0614	±0.0614	±0.0623	±0.0616	±0.0606	±0.0578
$\frac{r}{E_r}$	+2.77	−1.05	+1.15	−0.95	−0.78	+0.60	+0.11	+2.02	+4.28

masked by the effects of random sampling. Grouping all these pairs of determinations (140) and obtaining the correlation between protein in the flour (A-a) and blank (a) gave a coefficient of $+ .2172 \pm .0543$. with odds against occurrence due to random sampling of 140 to . This led to the thought that possibly there was a slight loss of nitrogen from both flour and chemicals, which was related to the individual heating characteristics of the burner. Series II was planned to throw further light upon this problem. The results apparently disprove the idea, for any effect of equipment should surely not be subject to such change during replicated series in one laboratory as to give changes in these correlations from $+.67$ to $-.35$. In all, the sign of the correlation is shown to be sufficiently inconsistent to render the average correlations for each collaborator insignificant despite significant individual correlations.

It might be contended from inspection of the disorderly signs of these correlation coefficients that the values have arisen by random sampling and that no real correlation exists in any case. If the correlations represent truly random samples from a population of paired total proteins and blanks showing no correlation, then the frequency distribution of the correlation coefficients should agree with the normal curve whose area is 58 units and whose standard deviation is given by

$$\sigma = \sqrt{20}\ (1-r^2) = \sqrt{20}$$

where r, based upon samples of 20 pairs, is zero. This curve is superimposed upon the histogram of the 58 correlation coefficients in Figure 7. While this diagram shows that even the highest correlation of $+.6754$ might have arisen in 58 samples from a population showing no correlation of the paired determinations, it is also apparent that the group of coefficients as a whole could not have so arisen. There is a marked deficiency in the correlations between $-.2$ and $+.1$ with corresponding excess below and above these values. Again, if the curve truly represented the distribution, then the average value of the ratios of the correlation coefficients to their probable errors (without regard to sign) should equal unity. Summing the ratios and dividing by 58, a value of 1.76 is obtained, leading to the conclusion that these coefficients are independently significant but show variation in magnitude and sign. The association between pairs of total proteins and blanks in the series of determinations reported by the collaborators cannot then be wholly attributed to random errors.

A definite association between two or more sequences of determinations of protein (upon the same sample in each series), such as is herein shown to exist, suggests a progressive variation in technic

which, when in the same sense in two series, will give a positive correlation between them and, *vice versa,* when in a reverse sense will give a negative correlation. In such a case, a positive correlation should be demonstrable between the individual determinations and the immediately succeeding ones of a series. The results of calculations to determine the existence of such correlations within the analyses of the second series of collaborators are submitted as Table VIII. It is well to point out that negative correlations by this procedure indicate alternation between a higher and a lower determination, and thus represent absence of progressive variation, whereas a positive correlation indicates that a determination varies or tends to vary with the one preceding it in the series. The variation might be in the same sense throughout the series or even change in sense one or more times. It will be apparent that each change of sense in progressive variation will diminish the correlation coefficient; in a limited series of 20 determinations very few such changes will make the coefficient negative. Thus the interpretation of the coefficients in Table VIII requires considerable caution. In all, they appear to indicate a tendency toward progressive variation for most analysts. This is the more marked with collaborators 14 and 16, while it is pronounced in the blank determinations of collaborator 9. Collaborator 12, however, shows a consistent tendency to alternate between a higher and a lower reading in these determinations.

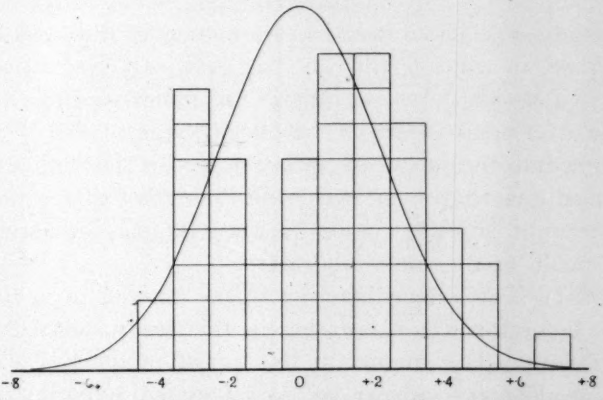

Fig. 7. Histogram of Correlations Between Paired Series of Protein Determinations The normal curve of error for zero correlation, based on samples of 20, is superimposed.

While it may not be possible to explain fully the correlations of Table VII by those represented in Table VIII, it does seem reasonable to conclude that the former may be attributable in some measure to progressive variation in technic of the analysts. Possibly this may appear in the titration of the excess acid, being due to changes of

color sensitivity of the retina of the analyst, especially in cases of failure to check against color standards in each titration. The differing sensitivity of human retina to color schemes is a commonly observable fact. The same analyst will frequently secure different end points with different indicators, while for the same reason different analysts working apart may fail to check each other when using the same indicator.

TABLE VIII

CORRELATIONS BETWEEN SUCCESSIVE DETERMINATIONS OF PROTEIN IN THE SAME SAMPLE

Collaborator	Flour *B*	Blank *b*	Flour *C*	Blank *c*
2	—0.1358	+0.3226	+0.2036	—0.0096
9	—0.2420	+0.6142	—0.0867	+0.7883
10	—0.0724	+0.1578	+0.1019	+0.6432
11	+0.1141	+0.0300	+0.0163	—0.4852
12	—0.2310	—0.2972	—0.2653	—0.2080
13	+0.1385	.....	+0.1892	—0.0474
14	+0.2180	+0.0950	+0.4345	+0.1806
15	—0.0353	—0.0326	+0.1515	—0.1223
16	—0.0882	+0.3420	+0.3452	+0.4624

The cause of the correlation between series of determinations of protein in flours and blanks made by the same analyst may remain obscure. The demonstration of the existence of such association is, however, clearly important in its bearing upon the general problem of the precision of analyses.

Summary

This study of collaborative protein testing in different laboratories of high repute, in which great care has been exercised to secure strict adherence to a standard method officially recommended by the American Association of Cereal Chemists, has been made primarily to obtain a better insight into the causes of variation in this determination, to subject to critical quantitative measurement the extent of the variation, and also to determine any association between comparable series of determinations made in the same laboratory.

Reasonable concordance appears in the amount of variation found within the laboratories collaborating in this study, with the submitted samples of flour. The amount of this variation changes with the flour sample, and does not appear to be associated with the quantity of protein present. It may be conditioned by the nature of the nuclei of the amino acids involved in the structure of the complex protein molecules.

Variation among the group of laboratories is approximately twice as great as that found within them individually, being due to the systematic personal or laboratory equation of analysts.

Definite standards of variation to be expected in protein analyses within and among laboratories characterized by the precision of those collaborating have been submitted. Analysts reporting the average of even three determinations in a protein test can be only reasonably sure that a repetition within their own laboratory would check within 0.2%, while for a single determination a replicate may fall more than 0.3% away. Checking against another laboratory of high repute using the same method, discrepancies of 0.35% for the average of 3 determinations, or even as high as 0.6% for single analyses, may be expected to arise.

That analysts systematically deviate in a definite direction so that highly significant and frequently very important differences arise between them in reporting the average value of replicate determinations has again become apparent. Furthermore, this study has indicated that analysts may show progressive variations in technic in making a series of determinations, these variations becoming apparent in the results of the analyses. Thus significant correlations appear between comparable series of determinations made in the same laboratory, and also between consecutive determinations of the same series.

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INDICATORS FOR AMMONIA TITRATIONS

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United States Department of Agriculture

(Read at the Convention, May, 1929)

Much careful work has been done by the American Association of Cereal Chemists and the Association of Official Agricultural Chemists with a view to developing a standard method for determination of nitrogen. Researches have been conducted in which variations have been made in the time of digestion, the heat applied, the quantity of sulphuric acid used, the substances employed to raise the boiling point of the sulphuric acid, the oxidizing agents and their precipitants, the standard acid used to hold the ammonia when distilled, the concentration of the standard alkali employed in titrating the excess of standard acid, and many other variables with the hope of eliminating all factors that might lead to discrepancies in results.

Since so much time and energy have been spent on the other factors, it seems proper that a little attention be given to the subject of indicators.

A review of the different reports fails to show that a comparative study has been made of indicators that are appropriate for use in titrating in the presence of ammonia.

Phelps and Daudt (1919) state that sodium alizarin sulphonate was used as indicator in their work, but make no further statements regarding it. Continuing the investigation the next year (1920) they used methyl red as indicator in hydrolyzing amins. Spears (1921) mentions the use of bromphenol blue as indicator, but makes no further comment.

Phelps, associate referee (1922), directed his collaborators to use methyl red indicator. The next year (1923) he gave directions to collaborators to use sodium alizarin sulphonate as indicator. Prince, associate referee (1925), states that methyl red was used as the indicator with the Devarda alloy method and sodium alizarin sulphonate with the Moore-Kjeldahl method.

Markley and Hann (1925) used sodium alizarin sulphonate with the Kjeldahl-Gunning-Arnold method and bromphenol blue with the boric acid method, but they made no comparisons of the respective

merits of the two indicators. They state that bromphenol blue may be used with artificial light and that a 4 per cent solution of boric acid is practically neutral to bromphenol blue.

Formerly the official method of the A.O.A.C. called for the use of cochineal indicator, but the present method directs that either cochineal or methyl red be used. The Methods of Analysis of Cereals and Cereal Products of the A.A.C.C. specify only methyl red as the indicator to be used in nitrogen determinations.

Now that the significance of hydrogen-ion concentration in connection with indicator phenomena is recognized, the pH values of the solutions concerned may be used to guide the selection of appropriate indicators. As some chemists use standard hydrochloric acid, and others standard sulphuric acid, to retain the ammonia distilled over, and as the end point of a titration should be taken as that at which the ammonium salts of these acids are formed, it is necessary to consider the pH values exhibited by both ammonium chloride and ammonium sulphate. Altho these salts are neutral in the stoichiometric sense, they are not neutral from the hydrogen-ion standpoint. The base being "weak" and the acid "strong," solutions of these salts contain an excess of hydrogen over hydroxyl ions and accordingly the reaction is acid. Actual trial of dilute solutions of the salts in freshly boiled distilled water showed: Ammonium chloride, pH 5.1; ammonium sulphate pH 5.7.

Of the many indicators that have been proposed, the following have useful ranges that cover the neutral points of ammonium chloride and ammonium sulphate from the hydrogen-ion standpoint. Litmus has a range of pH 4.6 to 8.4; both chlorphenol red and bromcresol purple have a range of pH 5.2 to 6.8; cochineal and sodium alizarin sulphonate, from pH 4.0 to 6.0; methyl red, from pH 4.4 to 6.0; and bromcresol green, from pH 4.0 to 5.6. From this list of indicators one must choose the most suitable. Litmus is the poorest of all, having too long a range and being unsatisfactory in the presence of ammonia. Chlorphenol red has a sharper color change, and the solution is more stable than bromcresol purple; both have the same pH range, but the final point at which color change is shown is pH 6.8, which is not sufficiently close to the pH of ammonium chloride or ammonium sulphate solutions. Cochineal solutions are not uniform, and difficulty is experienced at times in obtaining a supply of the material. It is believed that not many laboratories are using this indicator now.

Methyl red and sodium alizarin sulphonate have nearly the same range, and both are being used extensively.

Phelps (1923) points out that "when free sulphur is present in a Kjeldahl distillation of ammonia, sodium alizarin sulphonate and methyl red are troublesome indicators, but not so with cochineal."

Bromcresol green (tetra bromo-m-cresol sulphon-phthalein) was only recently synthesized by Dr. Barnett Cohen, of the Hygienic Laboratory of the United States Public Health Service. It has a useful range of 4.0 to 5.6, the final color change at pH 5.6 being near the pH of ammonium chloride and ammonium sulphate solutions, so that it is very desirable from that standpoint. Bromcresol green is yellow in an acid solution, changes to green as it approaches neutrality, and becomes blue in an excess of alkali. It is very sensitive, a single drop of a n/20 alkali solution changing its color from green to blue.

A comparison of color changes of bromcresol green and methyl red is shown in the following table:

pH	4.5	5.0	5.5	6.0
Bromcresol green	Yellow	Green	Blue	Blue
Methyl red	Red	Red	Orange	Yellow

Bromcresol green has nearly the same range as methyl red and may therefore be substituted for it. It also has the advantage of being more stable. It has an intermediate color, which gives a warning to the analyst as the end point is being approached.

Attention is called to this indicator that chemists may give it a trial, and if it proves satisfactory it may be adopted as an optional indicator in nitrogen work.

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EFFECT OF STORAGE AND OF VARIOUS BLEACHING AGENTS ON THE CAROTIN CONCENTRATION OF FLOUR[1]

CHARLES G. FERRARI AND C. H. BAILEY

Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station

(Received for publication September 1, 1928)

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The spontaneous oxidation of carotin in flour is a phenomenon that has long been recognized. It is frequently referred to as natural bleaching. It is not surprising that flour bleaches naturally, as, according to Bailey (1925, p. 198), approximately half the volume occupied by flour consists of air. The conditions for oxidation are, therefore, rather favorable than otherwise.

Winton (1911) noted the decrease in the gasoline color value of flours stored in air for thirty weeks. The gasoline color value decreased during this period to one third the original value.

Monier-Williams (1912) stored flour samples in air, exposing some of them to the open and sealing others in a closed tin. After two months the concentration of carotin in a sample stored in a closed tin decreased from 2.00 to 1.40 parts per million parts of flour; that in two exposed samples, from 2.00 to 1.12 parts.

Kent-Jones (1924, p. 175) confirmed the observation that oxidation is responsible for the natural bleaching of flour by storing straight grade flour for two months in a vacuum, in an atmosphere of hydrogen, and in air. The sample stored in a vacuum did not bleach; that stored in hydrogen showed little change, and that stored in air bleached normally.

The effect of direct sunlight on flour was reported by Avery (1907) and later by Shutt (1911). Each reported a hastening of the bleaching action.

In this study a sample of straight grade flour was obtained from the Minnesota State Testing Mill and aliquots were stored under different conditions. The control sample was stored on the laboratory shelf in a glass bottle with a rubber stopper. Beside it

[1] Published with the approval of the Director, as Paper No. 885, Journal Series, Minnesota Agricultural Experiment Station. Condensed from one section of a thesis presented by Charles G. Ferrari to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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The effect of direct sunlight on flour was reported by Avery (1907) and later by Shutt (1911). Each reported a hastening of the bleaching action.

In this study a sample of straight grade flour was obtained from the Minnesota State Testing Mill and aliquots were stored under different conditions. The control sample was stored on the laboratory shelf in a glass bottle with a rubber stopper. Beside it

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a second sample was stored in a glass jar in which the air had been replaced by carbon dioxide, and the rubber stopper paraffined. The method by which the air was replaced by carbon dioxide was as follows: The jar containing the flour sample was evacuated continuously for about an hour by the steady operation of a "Hyvac" pump. The vacuum was broken by allowing carbon dioxide from a cylinder to flow into the jar in place of air. The stopper with which the jar was fitted contained two glass tubes. The shorter tube just penetrated the stopper and was connected to the vacuum pump; the longer tube penetrated the flour sample and extended to the bottom of the jar. Carbon dioxide was passed through the latter. Exhaustion of the jar was repeated a number of times in the same manner. Finally carbon dioxide was allowed to flow through the jar slowly under atmospheric pressure for several hours. The tube arrangement described above made it necessary for the carbon dioxide to penetrate the flour, and in entering from the bottom and escaping from the top, carbon dioxide was continually sweeping out the gaseous contents of the jar.

A third sample was stored in air in a glass jar fitted with a rubber stopper, but was placed in a refrigerated room the temperature of which was maintained constant at 0° Centigrade.

The transmittancy of the gasoline extract of the control sample was observed at the beginning of the experiment, by the method previously outlined. At stated intervals the transmittancy of the gasoline extracts of all the samples was determined. In all cases 20 grams of sample was used with 100 cc. of high-test gasoline, and the transmittancy determined in a 10-cm. cell at a wave length of 435.8 mμ. The transmittancy data and the corresponding concentration of carotin in parts per million of flour are recorded in Table XIV.

Curves expressing the relation between the concentration of carotin and the number of days the sample was stored are shown in Figure 4.

The data show that low temperature had a greater effect in inhibiting the natural bleaching of flour than had an atmosphere of carbon dioxide. The rate at which the concentration of carotin decreased was not significantly different in the two samples stored at room temperature in air and in carbon dioxide. On the other hand, the concentration of carotin in the sample stored in air at 0° remained much higher. For example, the flour at the begin-

ning of the storage period had a carotin concentration of 2.46 parts per million parts of flour; at the end of 100 days, that stored in air at room temperature had a concentration of 1.85 parts per million of flour; that stored in carbon dioxide at room temperature, a concentration of 1.91 parts; and that stored at 0° in air, a concentration of 2.12 parts.

The plotted data (Fig. 4) show the temperature effect more graphically. The curve for the sample stored in air at 0°C. slopes off gradually at first and then shows a fairly uniform decrease in carotin concentration. This sample was stored at a constant temperature. On the other hand, the temperature at which the other samples were stored (room temperature) fluctuated. Altho one sample

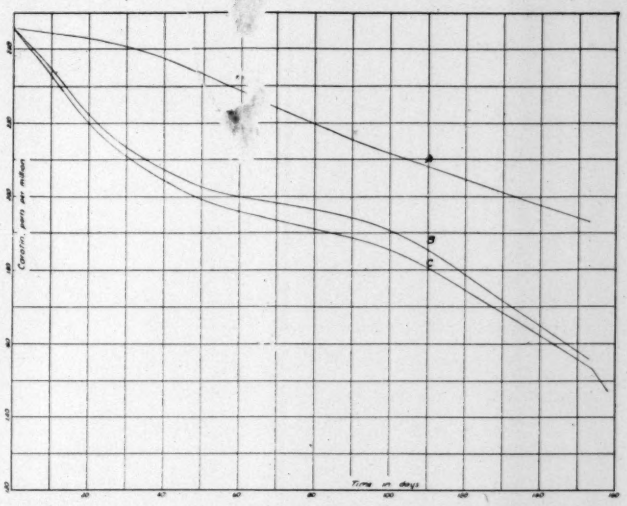

Fig. 4. Storage Experiment. Change in the Concentration of Carotin in Samples of Unbleached Straight Grade Flour Stored Under Different Conditions
A. Stored in air at 0°C.
B. Stored in CO_2 at room temperature
C. Control, stored in air at room temperature

was stored in air and the other in carbon dioxide, the curves parallel one another very closely, and have the same general shape. It appears that changes in the rate at which the concentration of carotin decreased in the samples stored in the laboratory were due largely to fluctuations in temperature, and the similarity of the curves indicates that carbon dioxide was not an important factor in influencing such changes.

TABLE XIV

EFFECT OF STORAGE CONDITIONS UPON CAROTIN CONCENTRATION OF FLOUR

Determined from transmittancy measurements of gasoline extracts of flour in a 10-cm. cell at a wave length of 435.8 mμ.

Control Sample Stored in Air at Room Temperature									
Days stored	0	5	16	29	31	72	100	154	158
Per cent transmittancy	11.2	11.6	12.0	16.0	17.4	18.3	18.8	25.7	27.2
Carotin, parts per million of flour	2.46	2.43	2.38	2.07	1.98	1.92	1.89	1.54	1.48
Sample Stored in Carbon Dioxide at Room Temperature									
Days stored	0	30	32	71	127	153			
Per cent transmittancy	11.2	15.1	15.3	15.4	18.8	25.0			
Carotin, parts per million of flour	2.46	2.14	2.13	2.12	1.89	1.57			
Sample Stored in Air at 0°C.									
Days stored	0	30	99	153					
Per cent transmittancy	11.2	11.6	15.1	17.8					
Carotin, parts per million of flour	2.46	2.43	2.14	1.95					

It might be argued that in the sample stored in carbon dioxide the replacement of air was incomplete. Insurance against error on this score was as great as ordinary laboratory facilities permitted. However, it is conceivable that air was held in the interior of the individual flour particles, and the method pursued for replacing this air was ineffectual. Oxidation of the carotin to leuco compounds could proceed even if the space between the flour particles had been successfully replaced by carbon dioxide. As considerable care was exercised in this operation it must be assumed that the air was replaced by carbon dioxide, and that the latter was not so effectual in inhibiting the oxidation of carotin as was low temperature.

Effect of Various Bleaching Processes on Carotinoid Pigment Content of Flour

Introduction.—Elsewhere in the discussion of flour color it was mentioned that the practice of bleaching was the direct outgrowth of the demand for white flour. The earliest record of the use of chemical substances to whiten flour is the British patent No. 2502, granted in 1879 to Beans (Bailey, 1925, 199). Almost twenty years elapsed before the commercial application of chemical bleaching was made, when Frichot, in 1898, was granted French and British patents, Nos. 277,751 and 21,971 respectively. Frichot thought his flaming arc generated ozone, which accomplished the bleaching. It was demonstrated some time later by Avery (1907) that the active agent was not ozone but nitrogen peroxide.

Snyder stated in 1904 that he was not aware at that time of any commercial application of the bleaching process in the United States. Having noted its introduction in Europe and its advocacy in this country he made some laboratory experiments in which he attempted to bleach flour with oxygen gas. About that time patents were granted Alsop (U. S. Patents Nos. 758,883; 758,884; and 759,651 issued in 1904) for the production of nitrogen peroxide by means of a flaming arc. The original use of nitrogen peroxide, however, was made by Andrews, in England, but he produced the gas by chemical means.

Since the introduction of nitrogen peroxide as a bleaching reagent many other substances have been used. Among the most prominent of these are chlorine; mixtures of chlorine with nitrosyl chloride; nitrogen trichloride; and organic peroxides of the type of benzoyl peroxide.

Nitrogen peroxide has the greatest historic interest as a bleaching reagent, as it was the first to be employed extensively on a commercial scale. The flour bleaching controversy hinging around the residual nitrites in flour after bleaching with this reagent, and the resulting agitation against all flour bleaching processes can be traced to it. It is commonly referred to in the United States as the Alsop process, and it is still used extensively as a commercial bleaching agent. The experiments reported here are not concerned with its use, chiefly because the application of other bleaching reagents was more convenient. Consequently the apparatus used and the reactions involved in nitrogen peroxide bleaching will not be discussed. A convenient summary is found in the "Chemistry of Wheat Flour" by Bailey (1925).

Among the substances mentioned as chemical bleaching agents, those used in this study were chlorine, nitrogen trichloride (known commercially as the Agene Process), and benzoyl peroxide in the form of the Novadel reagent. These will be discussed in the order mentioned.

Bleaching with chlorine gas.—The commercial use of chlorine as a flour bleaching reagent began about 1910 with a patent issued to Williams (No. 963,970). In the method of its application liquid chlorine is obtained in large cylinders. By using suitable valves and gauges the volume of gas discharged in a unit of time may be controlled. The dosage is usually expressed as ounces of chlorine per barrel of flour. The favorite manner of mixing the gas with the flour is by

the use of an agitator of some type. Agitation is essential in order not to overtreat one portion and ineffectually treat another.

The mechanism of the bleaching action of chlorine is of interest. The bleaching agent changes the carotin pigment to a colorless compound. Carotin is a highly unsaturated hydrocarbon of the formula $C_{40}H_{56}$. Its constitution is unknown. Its properties are not described in detail at this point as they were considered previously. In common with most unsaturated hydrocarbons, carotin reacts with the halogens. The first halogen derivative of carotin was described by Arnaud (1886) who prepared a derivative having the formula $C_{40}H_{56}I_3$. Willstätter and Escher (1910) prepared the same substance and in addition Willstätter and Mieg (1907) and Willstätter and Escher (1910) prepared a di-iodo derivative of the formula $C_{40}H_{56}I_2$, and a bromine compound of the formula $C_{40}H_{36}Br_{22}$. It will be noted that the latter is an addition compound as well as a substitution product.

It appears reasonable to suppose that chlorine addition products of carotin are formed in bleaching flour with chlorine. Nonpigmented compounds are formed, resulting in a flour with less yellow color. The extent to which the yellow color of flour is discharged depends upon the amount of chlorine used in the bleaching process.

Another explanation of the mechanism of the bleaching process is possible. The chlorine may combine with the water in the flour with the resulting formation of hypochlorous acid. The reduction of hypochlorous acid to hydrochloric acid follows and the oxygen released oxidizes the pigment. This reaction apparently takes place to some extent, as flour bleached with chlorine has been shown to have a greater acidity than untreated flour. Possibly chlorine addition compounds of carotin are formed, and also carotin oxidized by oxygen resulting from the reduction of hypochlorous acid. The carotin compounds formed in both cases are colorless.

Apparatus for laboratory application of gaseous bleaching agents.—In bleaching flour samples with chlorine in the laboratory the apparatus illustrated in Figure 5 was used.[2] It consisted of a wooden box (a) provided with a lid, which was made tight by the use of felt weather stripping. The charge of flour to be bleached was placed in this box. The box was provided with a handle (b) which permitted it to be rotated. A hollow tube (c) for the entrance of the bleaching gas was part of the bearing upon which the box was rotated, and extended to the middle of the box inside.

2 The apparatus for conducting the bleaching experiments, with the exception of the gas burettes, was furnished through the courtesy of the Wallace and Tiernan Company, Newark, N. J., to whom grateful acknowledgment is made.

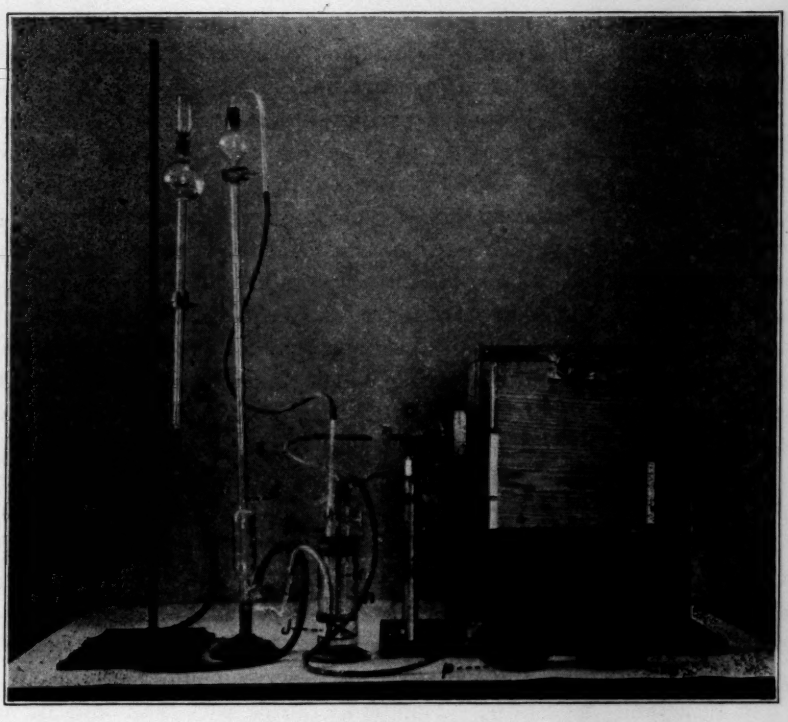

Fig. 5. Apparatus for Bleaching Flour with Chlorine and Nitrogen Trichloride, Largely Supplied by Wallace and Tiernan Company

A small cylinder of chlorine (d) was used to charge the gas burette (e) with the amount of chlorine it was desired to use. A leveling burette (f) completed the gas-measuring equipment.

In using the apparatus, the chlorine tank was connected to the gas inlet tube (g) of the gas burette, and after taking suitable precautions to insure that all the air in the connections was replaced by chlorine, an amount of chlorine in excess of the required amount was allowed to flow in, displacing the saturated aqueous solution of salt and chlorine. By adjusting the stopcock on the top of the gas burette and manipulating the leveling tube, the desired amount of chlorine was collected in the burette at atmospheric pressure. The bulb on top of the gas burette was then swept out with air to get rid of the chlorine that had been discharged into it. A glass tube was then firmly inserted in the top of the gas burette and connected to the glass cylinder (h) in the manner illustrated. This cylinder was provided with a porous diffusing disc (j) into which a silver tube (k) entered and passed at the top through a rubber stopper. The rubber stopper was also provided

with two glass tubes having glass stopcocks. The silver tube of the porous disc was connected by a rubber hose to a rubber bulb (m) with two valves and a constant pressure bulb (p).

Upon opening the proper stopcocks and raising the leveling bulb, the chlorine was allowed slowly to enter the cylinder (h), which was connected to the box through the tube (s) and the box containing the flour was rotated. Sufficient liquid from the leveling bulb was made to flow into the system to fill it to the end of the glass tube (r), which entered the cylinder. This insured a complete delivery of the gas into the cylinder. Pressure from the rubber bulb was applied gently and the chlorine swept gradually into the box. The cylinder was swept out with air for 20 minutes, during which time the box was rotated to mix the sample with the chlorine. At the end of 20 minutes it was assumed that all the chlorine had been transferred to the box containing the sample, and the sample was assumed to have been thoroly mixed with the bleaching gas.

Experimental results with chlorine bleaching.—In bleaching flour with chlorine it was customary to use 500 grams of flour whenever that quantity could be spared. The dosage was calculated as the basis of the number of cc. of chlorine per gram of flour to be bleached. The chlorine was carefully measured as described under the discussion of the bleaching apparatus. The temperature was read and likewise the barometric pressure. The volume of chlorine used was corrected to standard conditions of temperature and pressure. The corrected volume was multiplied by the weight of one cc. of chlorine at 0° C. and 760 mm. pressure, which in these calculations was taken to be 0.0031674 gram. From the grams of chlorine so computed the dosage of chlorine was calculated. It is reported as parts of chlorine per million parts of flour, and as ounces of chlorine per barrel of flour.

A sample of freshly milled straight grade flour (No. 400) was obtained from the Minnesota State Testing Mill, and subjected to the bleaching action of chlorine gas as described. Three dosages were employed, corresponding to 253, 315, and 394 parts of chlorine per million parts of flour. The same concentrations expressed as is customary in milling practice were, respectively, 0.8, 1.0, and 1.24 ounces of chlorine per barrel of flour. These ranges in concentration were chosen as representative of the dosages usually employed in commercial bleaching operations.

The transmittancy of the gasoline extracts of the flour was determined in the conventional manner, using 20 grams of sample with 100 cc. of gasoline and reading the transmittancy of the flour extract in a

10-cm. cell at a wave length of 435.8 mμ. From the transmittancy the concentration of carotin was ascertained from the curve expressing the relation between the per cent transmittancy and the concentration of carotin in petroleum ether solution. This method has been described previously.[3]

The transmittancy of the unbleached sample and of the bleached sample immediately after treatment was determined; and transmittancy measurements were made at intervals thereafter. The data secured are reported in Table XV. They show that a concentration of 253 parts of chlorine per million parts of flour bleached immediately about one half of the original concentration of carotin. In like manner concentrations of 315 and 394 parts of chlorine per million parts of flour bleached respectively about two thirds and three fourths of the carotin originally present in the sample. The flours stood in approximately the same ratio at the end of 104 days, when the proportion of carotin bleached was, in the smallest dosage of chlorine 66 per cent, next 71 per cent, and for the largest dosage 80 per cent.

TABLE XV

EFFECT OF CHLORINE TREATMENT OF STRAIGHT GRADE FLOUR NO. 400

Transmittancy determined in a 10-cm. cell at a wave length of 435.8 mμ.

Parts per million Oz. per bbl. flour	Chlorine dosage					
	253 0.795		315 0.989		394 1.236	
	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million
Unbleached flour — May 22	12.0	2.40	12.0	2.40	12.0	2.40
Immediately after bleaching	39.8	1.05	48.4	0.83	57.2	0.63
7 hours after bleaching	....	....	....	....	57.2	0.63
8 hours after bleaching	44.4	0.93	....	....	....	....
21 hours after bleaching	....	....	49.0	0.81	....	....
24 hours after bleaching	....	....	50.7	0.77	....	....
31 hours after bleaching	....	....	....	....	60.0	0.59
32 hours after bleaching	....	....	53.0	0.73	....	....
33 hours after bleaching	43.3	0.95	....	....	....	....
103 hours after bleaching	....	....	....	....	65.6	0.48
104 hours after bleaching	....	....	54.8	0.69	....	....
105 hours after bleaching	49.5	0.81	....	....	....	....
Control sample — July 2	13.4	2.28	13.4	2.28	13.4	2.28
Natural bleaching of control sample stored 105 hours (calculated)	....	0.013	....	0.013	....	0.013
Corresponding change in chlorine treated sample	....	0.24	....	0.14	....	0.15

The bleaching action of chlorine observed by conducting transmittancy measurements in this manner was in harmony with the results reported by Buck (1917), who reached practically identical conclusions from observations based on determinations of the gasoline color value

[3] Cereal Chem. 6:347-366 (1929).

of flour. He reported a bleaching effect with chlorine ranging from 64 to 71 per cent.

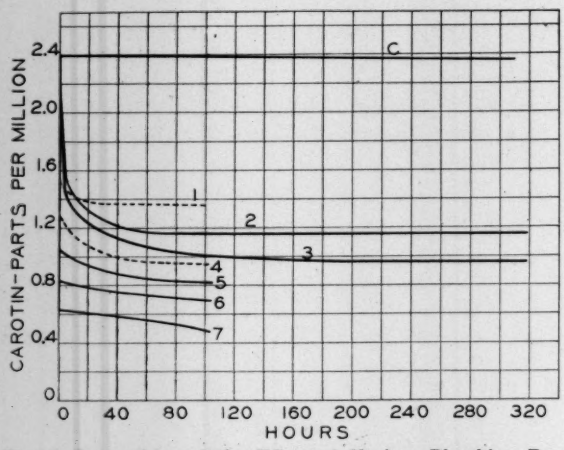

Fig. 6. Comparison of the Effect of Various Bleaching Reagents on Straight Grade Flour No. 400
C. Control sample
1. Sample treated with 15.9 parts of nitrogen trichloride per million parts of flour
2. Sample treated with 0.011% Novadel reagent
3. Sample treated with 0.0145% Novadel reagent
4. Sample treated with 47.8 parts of nitrogen trichloride per million parts of flour
5. Sample treated with 253 parts of chlorine per million parts of flour
6. Sample treated with 315 parts of chlorine per million parts of flour
7. Sample treated with 394 parts of chlorine per million parts of flour

Figure 6 shows the change in the concentration of carotin with time after straight grade flour No. 400 was treated with chlorine. Parts of carotin per million parts of flour are plotted along the ordinate. Time in hours is plotted along the abscissa. Other bleaching treatments of this sample are shown on the same graph and will be referred to later. The concentration of carotin determined immediately after bleaching is plotted as zero time, altho actually the flour was exposed to chlorine for 20 to 30 minutes before a sample was taken for examination. Examination of the curves will reveal that the carotin concentration of the control sample changed very slowly during the period of the experiment. Had the experiment been continued long enough the effect of the chlorine might be expected gradually to disappear until the rate at which the concentration of carotin changed was identical in both bleached and unbleached samples.

That the bleaching action of chlorine was not entirely an instantaneous process is significant. The greatest effect was produced at once, to be sure, but the bleaching action continued at a greater rate than can be accounted for by the natural bleaching of flour in storage. At the end of 105 hours, by calculation, the carotin concentration of the control sample changed only 0.013 part per million; that of the bleached samples decreased from 0.14 to 0.24 part of carotin per million of flour.

The Association of Operative Millers (1923) published the answers to the following question asked by one of their members: "Does chlorine continue to bleach flour after the flour is put in the package? In other words, if I am bleaching with chlorine and send out a fresh sample, and after that sample reaches destination a car is sold on the sample; and then say in twenty days after sample is drawn, we ship the car of flour, should the customer hold the sample and compare it with the flour sent out twenty days after sample was drawn, would the sample be whiter than the new-made flour?"

The answers to this question by five persons were reported and they are by no means in agreement. In all cases the answers were expressed as a matter of opinion.

R. S. Herman stated that a gradual change in color resulted upon storing flour treated with chlorine, but that the greatest change occurred at once. An anonymous writer and Edgar S. Miller agreed substantially with Herman, and stated that the rate of change in color in the chlorine-bleached flour was greater than could be ascribed to natural bleaching.

M. J. Blish and J. C. Wood expressed the opinion that the action of chlorine was instantaneous and that natural aging was the cause of further change in the color of the flour.

The data secured from transmittancy measurements reported here support the view that chlorine does continue to exert a bleaching effect for a long period after the treatment.

Another series of chlorine treatments was given to a sample of flour milled from Marquillo wheat. The bleaching of this sample was of particular interest owing to the high initial concentration of carotin it contained. The unbleached sample contained 4.56 parts of carotin per million parts of flour, almost twice as much as the sample of straight grade flour used in the experiment previously described.

The data secured with three dosages of chlorine are reported in Table XVI. It will be noted that the dosages applied bleached the same proportional amount of carotin that was bleached in the straight grade flour previously discussed. Immediately after bleaching a concentration of chlorine in the amounts of 254, 316, and 395 parts per million of flour bleached carotin equivalent to 51, 66, and 74 per cent respectively, of the original quantity. The data are plotted in Figure 7 along with curves for other bleaching treatments of the same flour

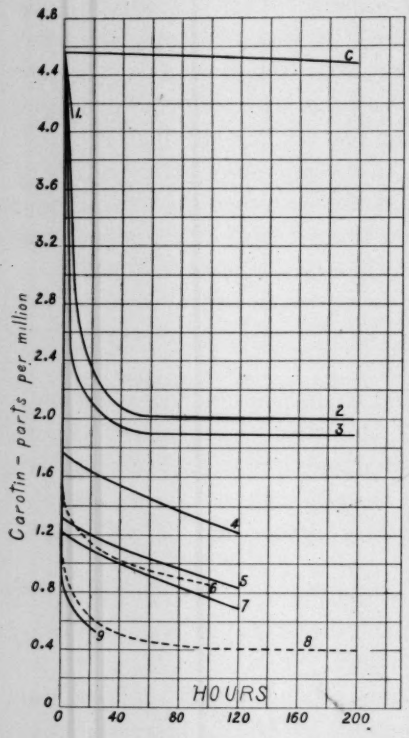

Fig. 7. Comparison of the Effect of Various Bleaching Reagents upon a Sample of Marquillo Wheat Flour

C. Control sample
1. Sample exposed to ultra-violet radiation
2. Sample treated with 0.0145% Novadel reagent
3. Sample treated with 0.016% Novadel reagent
4. Sample treated with 48 parts of nitrogen trichloride per million parts of flour
5. Sample treated with 64 parts of nitrogen trichloride per million parts of flour
6. Sample treated with 316 parts of chlorine per million parts of flour
7. Sample treated with 80 parts of nitrogen trichloride per million parts of flour
8. Sample treated with 395 parts of chlorine per million parts of flour
9. Sample treated with 96 parts of nitrogen trichloride per million parts of flour

discussed elsewhere. Once more the curves for the chlorine treatment show that the bleaching action of chlorine continues for an appreciable time after it has been applied, and is not entirely instantaneous, as has been supposed. It will be noted that the concentration of carotin immediately after bleaching is taken as zero time. Samples were taken as soon as agitation of the flour with the bleaching reagent was completed, but during this 20- to 30-minute period the bleaching reaction was going on. The initial concentration of 4.56 parts of carotin per million parts of flour is shown on the axis representing zero time to drop to the concentration determined immediately after bleaching with the various dosages of bleaching reagents. A comparison of these initial drops gives an indication of the immediate, relative, bleaching effect.

Bleaching with nitrogen trichloride.—Nitrogen trichloride is used as a commercial bleaching reagent under the name of the Agene process. This process was patented in 1921 by Baker. Nitrogen trichloride is an oily liquid, very highly explosive. Owing to its explosive property it is not an article of commerce, but in the method of its use is generated on the spot. The reaction for its preparation takes place in two stages as follows:

$$3\ Cl_2 + 3\ H_2O \rightleftharpoons 3\ HCl + 3\ HOCl.$$

$$3\ HOCl + NH_4Cl \rightarrow NCl_3 + 3\ H_2O + HCl.$$

TABLE XVI

EFFECT OF CHLORINE TREATMENT OF STRAIGHT GRADE FLOUR MILLED FROM A SAMPLE OF MARQUILLO WHEAT

Per cent transmittancy and concentration of carotin in the gasoline extract determined in a 10-cm. cell at a wave length of 435.8 mμ

	Chlorine dosage					
Per cent Parts per million Oz. per bbl. flour	0.0254 254 0.796		0.0316 316 0.990		0.0395 395 1.238	
	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million
Unbleached flour	1.8	4.56	1.8	4.56	1.8	4.56
Immediately after bleaching	14.0	2.23	25.4	1.55	35.7	1.17
24 hrs. after bleaching	....	....	44.9	0.92	59.8	0.59
100 hrs. after bleaching	....	....	40.4	1.03	69.5	0.41
70 days after bleaching	....	....	....	....	78.8	0.28
131 days after bleaching	....	....	....	....	78.8	0.28
Control sample after 131 days	5.9	3.21	5.9	3.21	5.9	3.21
Change in carotin concentration of control sample after 100 hrs.	....	....	....	0.0416	....	0.0416
Corresponding change in chlorine-treated flour stored 100 hours	....	....	....	0.52	....	0.76
Change in carotin concentration of control sample after 70 days	....	....	....	....	....	0.70
Corresponding change in chlorine-treated flour after 70 days	....	....	....	....	....	0.89

In the illustration given above, ammonium chloride was used. Either ammonium chloride or ammonium sulfate is satisfactory. The resulting nitrogen trichloride is removed from the liquid in which it is formed by blowing air through it. The gas that accomplished the bleaching is the nitrogen trichloride mixed with moist air.

Laboratory experiments for bleaching flour with nitrogen trichloride were conducted with the apparatus described under the section on chlorine bleaching. Somewhat different manipulation was necessary. An excess of ammonium chloride was placed in solution in the jar (h). (See Fig. 5.) The amount of chlorine it was necessary to use was estimated from the dosage of nitrogen trichloride desired. Then chlorine was measured out accurately in the gas burette and the volume corrected for temperature and pressure as previously described. From the weight of the chlorine used in the experiment, the weight of nitrogen trichloride formed was calculated from the equation for its preparation given above. The chlorine gas was transferred to the jar (h) and confined to it by turning off the appropriate stopcocks. The jar was shaken for several minutes in order to bring about the reaction for the formation of nitrogen trichloride. The jar was connected to the box containing the flour sample by means of the tube (s). Pressure on the bulb (m) caused air to be forced through the diffusing

disc (j). The air thus forced out volatilized and carried the nitrogen trichloride into the bleaching chamber where it was mixed with the flour by rotating the handle (b). Air was forced through the apparatus in this manner for approximately 20 minutes. Nitrogen trichloride is easily volatilized, and the procedure described adequately removes it from the jar (h) and transfers it completely to the box (a). The gas has a characteristic odor that is almost absent in the jar (h) at the end of the bleaching experiment.

Experimental results with nitrogen trichloride bleaching.—A sample of straight grade flour No. 400 from the Minnesota State Testing Mill was subjected to the bleaching action of nitrogen trichloride as previously described. Two dosages of the bleaching agent were used, namely, 15.9 and 47.8 parts per million parts of flour. In the commercial operation of this process it is customary to express the dosage as grams of nitrogen trichloride per barrel of flour. Expressed on this basis the dosage was 1.42 and 4.25 grams per barrel, respectively.

The bleaching action of nitrogen trichloride was similar to that of chlorine in that it was not instantaneous. This is shown by the data given in Table XVII. Immediately after bleaching with 15.9 parts of nitrogen trichloride per million parts of flour, the concentration of carotin in the flour was reduced 36 per cent. At the end of 101 hours 43 per cent of the carotin had been bleached. In the sample treated with 47.8 parts of nitrogen trichloride per million of flour, the carotin content was reduced 47 per cent immediately after bleaching, and 60 per cent 102 hours later.

The data in Table XVII are depicted graphically in Fig. 6 along with other data described elsewhere. The concentration of carotin is plotted along the axis of ordinate and time is plotted along the abscissa. The curves show the rate at which carotin concentration changed with time after the sample was bleached. The rate of change was much greater for the first 30 hours than thereafter, when it approximated more closely the change occurring in the natural bleaching of flour. This was exactly what might be expected, as with the lapse of time the concentration of the gas retained by the flour becomes less, both because it is removed from the field of action by reacting with the pigment, and also because it would diffuse out of the flour.

A similar bleaching experiment with nitrogen trichloride was conducted, using Marquillo wheat flour. As indicated above, this flour is of special interest because the concentration of carotin in it is greater than was observed in any other sample. For this reason the dosages of nitrogen trichloride used were increased, ranging from 48 to 96

parts per million of flour. This concentration corresponds to a range of 4.26 to 8.53 grams of nitrogen trichloride per barrel of flour.

TABLE XVII

EFFECT OF BLEACHING STRAIGHT GRADE FLOUR NO. 400 WITH NITROGEN TRICHLORIDE AS SHOWN BY THE PER CENT TRANSMITTANCY OF GASOLINE EXTRACTS AND THE CONCENTRATION OF CAROTIN IN PARTS PER MILLION PARTS OF FLOUR

Determined in a 10-cm. cell at a wave length of 435.8 mμ.

	Dosage of nitrogen trichloride			
Parts per million	15.9		47.8	
Grams per bbl. flour	1.42		4.25	
	Per cent transmittancy	Carotin, parts per million	Percent transmittancy	Carotin, parts per million
Unbleached flour	12.0	2.40	12.0	2.40
Immediately after bleaching	25.9	1.53	32.3	1.28
5 hours after bleaching	....	....	33.9	1.23
18 hours after bleaching	25.9	1.53	36.2	1.15
29 hours after bleaching	29.7	1.37	....	....
30 hours after bleaching	....	....	42.4	0.97
101 hours after bleaching	30.0	1.36	....	....
102 hours after bleaching	....	....	43.4	0.95
Control sample after 101 hours	12.1	2.39	12.1	2.39
Change in carotin concentration of control sample after 101 hours	....	0.01	....	0.01
Change in carotin concentration of bleached flour after storing 101 hours	....	0.17	....	0.33

The transmittancies of the gasoline extracts were determined in the conventional manner and read immediately after the bleaching operation and at intervals thereafter. The data are reported in Table XVIII, and are depicted graphically in Figure 7. A progressive change in the bleaching action was again noted with the lapse of time, the rate of change being much greater than occurred in the unbleached sample stored for the same time. In the sample bleached with 48 parts of nitrogen trichloride per million of flour, the carotin concentration was reduced 73 per cent in 5 days. In like manner, in the sample bleached with 64 and 80 parts of nitrogen trichloride per million of flour, the reduction in carotin concentration was 81 and 85 per cent, respectively. The sample treated with 96 parts of nitrogen trichloride per million of flour was bleached 96% at the end of 71 days. The latter constituted an almost complete bleaching, as the amount of carotin found at the end of this time was only 0.16 part per million parts of flour. The gasoline extract appeared colorless to the eye.

The curves in Figure 7 illustrate the bleaching effect of nitrogen trichloride and other reagents on Marquillo wheat flour. Time on the abscissa is plotted against concentration of carotin on the ordinate. For the time interval for which the curves are drawn, it will be noted that the curve for the control sample is almost horizontal, indicating a scarcely appreciable change in the concentration of carotin; while the

curves for the bleached samples slope considerably. It is interesting to note that with dosages of 48, 64, and 80 parts of nitrogen trichloride per million of flour, the rates at which carotin concentration changed were almost identical, as is indicated by the similarity in the slopes of the respective curves. After storing for 5 days the change in carotin concentration was almost identical in samples bleached with 48, 64, and 80 parts of nitrogen trichloride per million of flour. This seems rather significant, as in the last case almost twice the quantity of the bleaching reagent was used as in the first. To be specific, in samples bleached with 48, 64, and 80 parts of nitrogen trichloride per million of flour the change in carotin concentration after storing the bleached samples for 5 days was 0.56, 0.48, and 0.53 part of carotin per million of flour. During the corresponding period the control sample changed 0.04 part of carotin per million of flour.

TABLE XVIII

EFFECT OF BLEACHING MARQUILLO WHEAT FLOUR WITH VARYING DOSAGES OF NITROGEN TRICHLORIDE AS SHOWN BY THE PER CENT TRANSMITTANCY OF THE GASOLINE EXTRACT, AND THE CONCENTRATION OF CAROTIN IN PARTS PER MILLION OF FLOUR

Determined in a 10-cm. cell at a wave length of 435.8 mμ.

Parts per million Grams per bbl. flour	Dosage of nitrogen trichloride							
	48 4.26		64 5.68		80 7.11		96 8.53	
	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million
Unbleached flour	1.8	4.56	1.8	4.56	1.8	4.56	1.8	4.56
Immediately after bleaching	20.8	1.78	31.2	1.32	34.3	1.22	41.7	1.00
1 day after bleaching	24.0	1.62	30.6	1.35	34.5	1.21	63.7	0.52
5 days after bleaching	34.2	1.22	48.1	0.84	54.4	0.69	....	....
71 days after bleaching	....	....	....	....	....	....	87.6	0.16
Control sample after 5 days	1.86	4.52	1.86	4.52	1.86	4.52	1.86	4.52
Change in carotin concentration of control sample after 5 days	....	0.04	....	0.04	....	0.04	....	0.04
Change in carotin concentration of bleached flour after storing 5 days	....	0.56	....	0.48	....	0.53	....	....
Change in carotin concentration of bleached flour after storing 71 days	....	....	....	....	....	....	....	0.84

Bleaching with benzoyl peroxide.—Benzoyl peroxide is the organic peroxide used most extensively as a flour bleaching reagent. It is marketed under the name of the Novadel reagent, or Novadelox, and in this form consists of 1 part of benzoyl peroxide and 3 or 5 parts of calcium phosphate. Its use as a flour bleaching reagent is fairly recent. It was introduced into the United States in approximately 1920. In the data reported here, the dosage is always expressed on the

basis of the Novadel reagent and not on the basis of the peroxide itself. Patents have been granted involving the use of this peroxide as a flour bleaching reagent, chiefly under the following names: H. C. J. H. Gelissen, T. Kroeber, Karl Ludecke, Ralph H. McKee, and E. C. Sutherland.

Dibenzoyl peroxide may be prepared by the reaction of benzoyl chloride with barium peroxide in the presence of water. The reaction may be expressed as follows:

$$2\,C_6H_5{-}\overset{\overset{O}{\|}}{C}{-}Cl + BaO_2 \xrightarrow{H_2O} C_6H_5{-}\overset{\overset{O}{\|}}{C}{-}O{-}O{-}\overset{\overset{O}{\|}}{C}{-}C_6H_5 + BaCl_2.$$

It may also be prepared by benzoylating hydrogen peroxide according to the Schotten-Baumann reaction. In this reaction 10 per cent hydrogen peroxide is cooled and treated with the necessary quantity of sodium hydroxide and benzoyl chloride. The mixture is shaken until no more precipitate forms and the smell of benzoyl chloride has disappeared. The peroxide is then recrystallized from boiling alcohol, 70 per cent of the theoretical amount being yielded.

Dibenzoyl peroxide results when a mixture of benzaldehyde and acetic anhydride are stirred together. This reaction requires several days and the materials are usually mixed with sand and allowed to stand. When the sand is extracted with ether and the ether evaporated spontaneously, a residue is formed that consists of 80 per cent of the theoretical amount of benzoyl peroxide. This reaction has been written in several ways. According to Erlenmeyer, ozone is formed that oxidizes the benzaldehyde to benzoyl peroxide. Another suggested reaction mechanism is the formation of benzoic acid from benzaldehyde and the subsequent reaction with acetic anhydride to form benzoic anhydride. The latter is converted to the peroxide by oxidation. Benzaldehyde and benzoic anhydride react to form dibenzoyl peroxide, and sodium peroxide has been used with benzoyl chloride.

Dibenzoyl peroxide is now manufactured in the United States by the first reaction described, that is, by the use of benzoyl chloride and barium peroxide.

Dibenzoyl peroxide crystallizes in the rhombic system. Its melting point is 103.5°C., but very little can be melted without decomposition. It is soluble in ether, benzene, and carbon bisulfide, and is somewhat soluble in alcohol.

The reactions of dibenzoyl peroxide have been described by Gelissen in a series of papers appearing in "Die Berichte der Deutsche chemische Gesellschaft" from 1925 to date. In many of the reactions that dibenzoyl peroxide undergoes, benzoic acid is a by-product. No

one has ever demonstrated the existence of benzoic acid in the flour after bleaching with this reagent, but it is reasonable to suppose that it is present. The extremely small amount of peroxide used in bleaching with the resulting minute amount of benzoic acid residue in the flour has made it difficult to detect.

Among the properties of dibenzoyl peroxide that may be of interest in connection with its use as a flour bleaching agent may be mentioned its explosive properties. It explodes on heating and on contact with sulfuric acid. Mixed with calcium phosphate under the name of the Novadel reagent it becomes apparently safe to ship and to handle in a flour mill.

Dibenzoyl peroxide differs from other bleaching reagents in being a solid substance. It was formerly the practice to feed it into the stock coming from the first break rolls. The preferred practice now is to treat the finished flour in an efficient agitator. A special feeder introduces the desired dosage mechanically into the flour stream. It is customary to use one pound of the Novadel reagent to 35-40 barrels of flour. In the experiments reported here, one pound to 30, 35, and 40 barrels of flour was used. It corresponds to 0.017 per cent, 0.0146 per cent, and 0.0128 per cent, respectively, of the commercial reagent. As peroxide forms only about 25 per cent of the Novadel reagent, it must be remembered that the active bleaching reagent constitutes a fraction of the above amounts.

The method for bleaching flour used in the laboratory was to weigh 500 grams of flour into a large glass jar. The Novadel reagent in the desired amount was weighed carefully on an analytical balance and introduced into the jar. The jar was provided with a rubber stopper and was shaken from end to end and simultaneously rotated for 20 minutes. In the data recorded in the tables under "Immediately after bleaching," the sample was taken at the end of this 20-minute agitation. The peroxide had an opportunity to act during the mixing.

Some data secured in bleaching experiments with the Novadel reagent are recorded in Table XIX. A sample of straight grade flour (No. 100) was bleached with 0.011, 0.0145, and 0.016 per cent of the Novadel reagent. As might be expected from the fact that dibenzoyl peroxide is a solid substance, the bleaching action is much slower compared to the action of gaseous bleaching reagents. At the end of 4 hours percentages of 0.011, 0.0145, and 0.016 bleached respectively 24, 38, and 41 per cent of the original carotin concentration of the flour as determined from transmittancy measurements conducted on gasoline extracts as previously described. At the end of 153 hours,

approximately 60 per cent of the carotin originally present was bleached in all the dosages used.

The data are plotted in Figure 8, in which the co-ordinates are hours versus carotin concentration in parts per million parts of flour. The transmittancy of the gasoline extracts of all samples was determined immediately after bleaching, and the rate of change in transmittancy

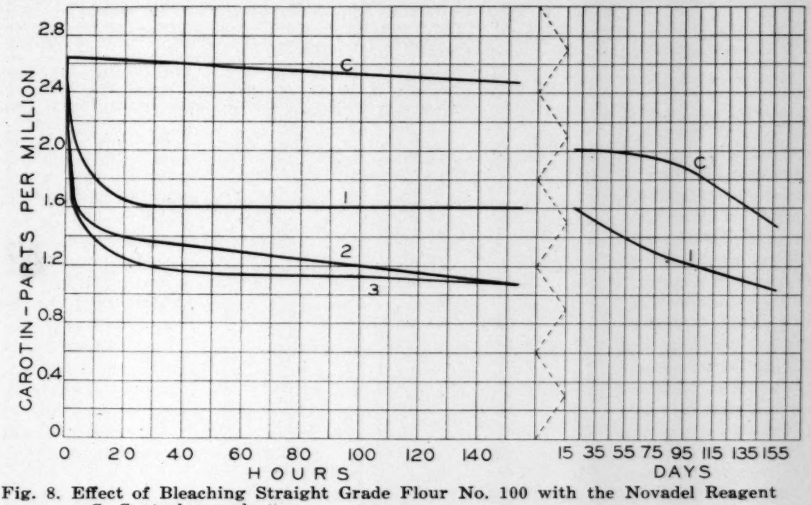

Fig. 8. Effect of Bleaching Straight Grade Flour No. 100 with the Novadel Reagent
C. Control sample
1. Sample treated with 0.011% Novadel reagent
2. Sample treated with 0.0145% Novadel reagent
3. Sample treated with 0.016% Novadel reagent

was followed by making observations at regular intervals. The curves show at first a fairly rapid decrease in the concentration of carotin with the lapse of time. At approximately 20 hours after bleaching the curves flatten out. This indicates a relatively smaller diminution of carotin concentration with lapse of time.

The data for the control sample, recorded in the tables and shown in the graph, are calculated values based on observations taken at a longer time interval than the limit of the experiments. As the carotin concentration changes in the control sample very slowly, the calculated data should not be much in error.

In the sample bleached with 0.011 per cent, observations were continued up to 158 days after bleaching. At the end of this time, 61% of the original quantity of carotin was bleached. A bleaching effect of equal intensity was secured with the larger dosages of the Novadel reagent in as many hours. At the end of 30 to 40 hours the maximum bleaching effect was secured; thereafter the rate of change in carotin

concentration was slower, with one exception, than in the control sample. The reverse was observed in flour bleached with gaseous reagents.

TABLE XIX

EFFECT OF BLEACHING STRAIGHT GRADE FLOUR NO. 100 WITH THE NOVADEL REAGENT (DIBENZOYL PEROXIDE) AS SHOWN BY THE PER CENT TRANSMITTANCY OF THE GASOLINE EXTRACTS AND THE CONCENTRATION OF CAROTIN IN PARTS PER MILLION OF FLOUR

Determined in a 10-cm. cell at a wave length of 435.8 mμ.

	Unbleached sample	Dosage of Novadel reagent					
		0.011%		0.0145%		0.016%	
	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million
Unbleached flour	2.65	9.6	2.65	9.6	2.65	9.6	2.65
Immediately after bleaching	.....	11.0	2.50	12.6	2.34	13.0	2.31
1 hr. after bleaching	.....	13.5	2.27	16.4	2.05	18.3	1.93
2 hrs. after bleaching	....	....	....	20.3	1.80	23.6	1.64
3 hrs. after bleaching	....	16.6	2.04	23.9	1.62	....	....
4 hrs. after bleaching	.....	.....	....	24.9	1.58	25.1	1.56
4½ hrs. after bleaching	.....	17.0	2.00	.....	.....	.....	.....
27 hrs. after bleaching	2.63	.....	.....	29.9	1.37	34.9	1.20
31 hrs. after bleaching	2.63	24.3	1.60	.....	.....	.....	.....
59 hrs. after bleaching	2.57	.....	.....	32.7	1.27	.....	.....
104 hrs. after bleaching	2.53	.....	.....	.....	.....	37.5	1.12
105 hrs. after bleaching	2.53	.....	.....	34.9	1.20	.....	.....
129 hrs. after bleaching	2.50	.....	.....	.....	.....	37.7	1.11
130 hrs. after bleaching	2.50	.....	.....	37.6	1.11	.....	.....
153 hrs. after bleaching	2.47	.....	.....	.....	.....	38.3	1.09
154 hrs. after bleaching	2.47	.....	.....	39.1	1.07	.....	.....
30 days after bleaching	2.01	24.4	1.62	.....	.....	.....	.....
70 days after bleaching	1.96	31.4	1.31	.....	.....	.....	.....
97 days after bleaching	1.88	34.4	1.22	.....	.....	.....	.....
158 days after bleaching	1.47	40.8	1.03	.....	.....	.....	.....

Another sample of straight grade flour (No. 400) was bleached with 0.011 and 0.0145 per cent of the Novadel reagent. The results obtained from transmittancy measurements of gasoline extracts were similar to those reported for sample No. 100. At the end of six hours, 35 per cent of the carotin had been bleached with the lighter treatment, and 44 per cent with the heavier. At the end of 80 hours the carotin concentration had decreased to 50 per cent of its original quantity in the sample bleached with 0.011 per cent of the reagent and 60 per cent was bleached in the sample treated with 0.0145 per cent. These data are recorded in Table XX.

The data are depicted graphically in Figure 6. Time, in hours, is plotted along the abscissa and carotin concentration in parts per million of flour along the axis of ordinate. These curves show the rate at which the concentration of carotin decreased with the lapse of time. A curve for the control sample is given for comparison.

Observations made on the transmittancy of the gasoline extracts of the samples during the period from 80 to 318 hours failed to reveal

a greater change in carotin concentration in the bleached samples than was shown by the control sample. The data for the control sample were calculated as previously described.

TABLE XX

EFFECT OF BLEACHING STRAIGHT GRADE FLOUR NO. 400 WITH THE NOVADEL REAGENT (DIBENZOYL PEROXIDE) AS SHOWN BY THE PER CENT TRANSMITTANCY OF THE GASOLINE EXTRACTS AND THE CONCENTRATION OF CAROTIN IN PARTS PER MILLION OF FLOUR

Determined in a 10-cm. cell at a wave length of 435.8 mμ.

	Unbleached sample	Sample treated with 0.011% Novadel reagent		Sample treated with 0.0145% Novadel reagent	
	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million
Unbleached flour	2.40	12.0	2.40	12.0	2.40
Immediately after bleaching	....	12.3	2.38	15.0	2.14
1 hour after bleaching	....	15.2	2.14	18.3	1.93
2 hours after bleaching	....	20.5	1.79	19.0	1.88
3 hours after bleaching	....	22.7	1.68	24.7	1.58
4 hours after bleaching	....	24.7	1.58	....	....
5 hours after bleaching	....	....	....	27.5	1.47
6 hours after bleaching	....	25.2	1.56	30.7	1.34
7 hours after bleaching	....	27.1	1.47	....	....
55 hours after bleaching	2.39	....	....	32.0	1.29
80 hours after bleaching	2.38	....	....	42.9	0.96
81 hours after bleaching	2.38	35.8	1.17	....	....
318 hours after bleaching	2.36	....	....	42.6	0.97
319 hours after bleaching	2.36	34.9	1.19	....	....

Another sample of straight grade flour, No. 600, was bleached with the Novadel reagent primarily to learn more, if possible, concerning the rate of change in carotin concentration after 12 hours, and also to observe the effect of an increased dosage of the reagent. The data are reported in Table XXI, and the results are shown graphically in Figure 9.

TABLE XXI

EFFECT OF BLEACHING STRAIGHT GRADE FLOUR NO. 600 WITH THE NOVADEL REAGENT (DIBENZOYL PEROXIDE) AS SHOWN BY THE PER CENT TRANSMITTANCY OF THE GASOLINE EXTRACTS AND THE CONCENTRATION OF CAROTIN IN PARTS PER MILLION OF FLOUR

Determined in a 10-cm. cell at a wave length of 435.8 mμ.

	Unbleached sample	Sample treated with 0.0145% Novadel reagent		Sample treated with 0.017% Novadel reagent	
	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million
Unbleached flour	2.32	12.8	2.32	12.8	2.32
Immediately after bleaching	....	18.1	1.94	18.2	1.93
1 hr. after bleaching	....	22.4	1.69	22.6	1.68
13 hrs. after bleaching	....	28.9	1.40	33.7	1.23
15 hrs. after bleaching	....	30.3	1.35	34.3	1.22
18 hrs. after bleaching	....	32.5	1.27	35.6	1.17
23 hrs. after bleaching	2.32	35.4	1.18	36.5	1.14
26 hrs. after bleaching	2.31	36.5	1.14	39.1	1.06
97 hrs. after bleaching	2.30	39.7	1.05	....	....
98 hrs. after bleaching	2.30	....	....	45.0	0.91

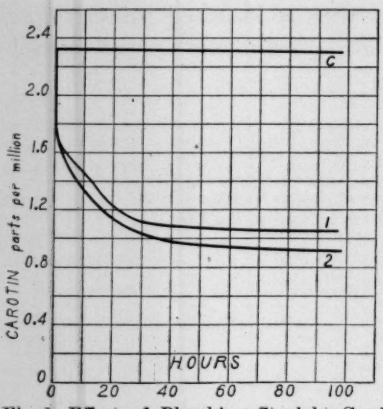

Fig. 9. Effect of Bleaching Straight Grade Flour No. 600 with the Novadel Reagent
C. Control sample
1. Sample treated with 0.0145% Novadel reagent
2. Sample treated with 0.017 % Novadel reagent

It appears from the data that the greatest bleaching effect took place in the neighborhood of 25-30 hours, after which the rate rapidly declined. However, at the end of 25 hours the carotin concentration decreased with both dosages at a greater rate than in the control sample.

At the end of 23 hours, the carotin concentration in the samples bleached with 0.0145 and 0.017 per cent of the Novadel reagent decreased 49 and 51 per cent respectively. At the end of 26 hours, 51 and 54 per cent of the carotin originally present was bleached, and at the end of 98 hours, 55 and 61 per cent. These data agree roughly with data previously reported for other samples that were bleached with similar concentrations of the Novadel reagent.

A sample of Marquillo wheat flour was bleached with 0.0145 and 0.016 per cent of the Novadel reagent. As has been stated, this flour is characterized by having a very high carotin concentration, and bleaching experiments conducted with it have, therefore, special interest. The samples were treated as previously described, by agitating the 500 grams of flour for 20 minutes in a glass jar with the carefully weighed quantity of Novadel reagent. The per cent transmittancy of the gasoline extract of the sample was determined in the usual manner at regular intervals. The data are recorded in Table XXII. At the end of 5 hours, 0.0145 per cent of the Novadel reagent bleached 29 per cent of the original amount of carotin in the sample, and at the end of the same time 0.016 per cent of the reagent bleached 47 per cent of the carotin. The bleaching action continued fairly rapidly until at the end of 52 hours a 57 per cent bleach had been effected with the smaller dosage, and a 60 per cent bleach with the larger. No appreciable change in carotin concentration occurred from 52 hours after bleaching to 197 hours. The data show that the control sample changed more during this period than the samples which were bleached.

TABLE XXII

EFFECT OF BLEACHING MARQUILLO WHEAT FLOUR WITH THE NOVADEL REAGENT (DIBENZOYL PEROXIDE) AS SHOWN BY THE PER CENT TRANSMITTANCY OF THE GASOLINE EXTRACTS AND THE CONCENTRATION OF CARTOIN IN PARTS PER MILLION OF FLOUR

Determined in a 10-cm. cell at a wave length of 435.8 mμ.

	Unbleached sample	Sample treated with 0.0145% Novadel reagent		Sample treated with 0.016% Novadel reagent	
	Carotin, parts per million	Per cent trans-mittancy	Carotin, parts per million	Per cent trans-mittancy	Carotin, parts per million
Unbleached flour	4.56	1.8	4.56	1.8	4.56
Immediately after bleaching	....	....	....	2.1	4.39
5 hours after bleaching	....	5.3	3.34	10.9	2.50
52 hours after bleaching	4.63	16.8	2.02	18.7	1.90
197 hours after bleaching	4.47	17.0	2.00	18.9	1.89
1176 hours (49 days) after bleaching	....	20.8	1.78	19.9	1.84

The data are plotted in Figure 7 as previously described. It is of special interest to note that the curves are practically perpendicular to the axis of the ordinate after the 52 hours, indicating scarcely a change in carotin concentration. The rate at which the control sample changed in carotin content during the period of this experiment is indicated at the top of the graph. The change appeared to be just as slow in the Marquillo wheat flour as in the other flours.

In reviewing the data on samples bleached with the Novadel reagent, it seems significant that all the flours, with their different initial carotin concentrations, are bleached about the same proportional amount in the same length of time. In all samples the original carotin bleached in 5 hours with 0.011 per cent of the Novadel reagent was approximately 29 per cent. In the period from 153 to 197 hours, all samples with all dosages of Novadel reagent approximated a 60 per cent bleach of the original amount of carotin present. While larger dosages of Novadel appeared to yield a greater bleaching effect in the shorter intervals of time, the final bleaching effect after a long interval of time was almost equalized. The flours bleached with the larger dosages of the Novadel reagent did not maintain their advantage over smaller dosages after a comparatively long time.

Bleaching with ultra-violet radiation.—The effect of sunlight in bleaching flour has long been recognized, but few data have been reported in the literature giving quantitative results after definitely timed exposures. Shutt (1911) placed flour one-quarter inch thick between glass plates and exposed it to sunlight. He stated that a bleaching was noticed at the end of one hour. He did not, however, report gasoline color values and he appears to have reached his conclusions merely by observing the visual appearance. As the effect of sunlight in bleaching flour is probably due chiefly to the ultra-violet rays, it

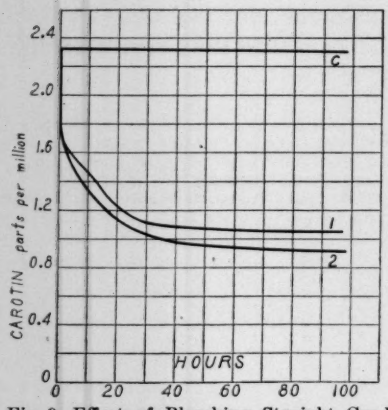

Fig. 9. Effect of Bleaching Straight Grade Flour No. 600 with the Novadel Reagent
C. Control sample
1. Sample treated with 0.0145% Novadel reagent
2. Sample treated with 0.017 % Novadel reagent

It appears from the data that the greatest bleaching effect took place in the neighborhood of 25-30 hours, after which the rate rapidly declined. However, at the end of 25 hours the carotin concentration decreased with both dosages at a greater rate than in the control sample.

At the end of 23 hours, the carotin concentration in the samples bleached with 0.0145 and 0.017 per cent of the Novadel reagent decreased 49 and 51 per cent respectively. At the end of 26 hours, 51 and 54 per cent of the carotin originally present was bleached, and at the end of 98 hours, 55 and 61 per cent. These data agree roughly with data previously reported for other samples that were bleached with similar concentrations of the Novadel reagent.

A sample of Marquillo wheat flour was bleached with 0.0145 and 0.016 per cent of the Novadel reagent. As has been stated, this flour is characterized by having a very high carotin concentration, and bleaching experiments conducted with it have, therefore, special interest. The samples were treated as previously described, by agitating the 500 grams of flour for 20 minutes in a glass jar with the carefully weighed quantity of Novadel reagent. The per cent transmittancy of the gasoline extract of the sample was determined in the usual manner at regular intervals. The data are recorded in Table XXII. At the end of 5 hours, 0.0145 per cent of the Novadel reagent bleached 29 per cent of the original amount of carotin in the sample, and at the end of the same time 0.016 per cent of the reagent bleached 47 per cent of the carotin. The bleaching action continued fairly rapidly until at the end of 52 hours a 57 per cent bleach had been effected with the smaller dosage, and a 60 per cent bleach with the larger. No appreciable change in carotin concentration occurred from 52 hours after bleaching to 197 hours. The data show that the control sample changed more during this period than the samples which were bleached.

TABLE XXII

EFFECT OF BLEACHING MARQUILLO WHEAT FLOUR WITH THE NOVADEL REAGENT (DIBENZOYL PEROXIDE) AS SHOWN BY THE PER CENT TRANSMITTANCY OF THE GASOLINE EXTRACTS AND THE CONCENTRATION OF CARTOIN IN PARTS PER MILLION OF FLOUR

Determined in a 10-cm. cell at a wave length of 435.8 mμ.

	Unbleached sample	Sample treated with 0.0145% Novadel reagent		Sample treated with 0.016% Novadel reagent	
	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million
Unbleached flour	4.56	1.8	4.56	1.8	4.56
Immediately after bleaching	....	.....	....	2.1	4.39
5 hours after bleaching	....	5.3	3.34	10.9	2.50
52 hours after bleaching	4.63	16.8	2.02	18.7	1.90
197 hours after bleaching	4.47	17.0	2.00	18.9	1.89
1176 hours (49 days) after bleaching	....	20.8	1.78	19.9	1.84

The data are plotted in Figure 7 as previously described. It is of special interest to note that the curves are practically perpendicular to the axis of the ordinate after the 52 hours, indicating scarcely a change in carotin concentration. The rate at which the control sample changed in carotin content during the period of this experiment is indicated at the top of the graph. The change appeared to be just as slow in the Marquillo wheat flour as in the other flours.

In reviewing the data on samples bleached with the Novadel reagent, it seems significant that all the flours, with their different initial carotin concentrations, are bleached about the same proportional amount in the same length of time. In all samples the original carotin bleached in 5 hours with 0.011 per cent of the Novadel reagent was approximately 29 per cent. In the period from 153 to 197 hours, all samples with all dosages of Novadel reagent approximated a 60 per cent bleach of the original amount of carotin present. While larger dosages of Novadel appeared to yield a greater bleaching effect in the shorter intervals of time, the final bleaching effect after a long interval of time was almost equalized. The flours bleached with the larger dosages of the Novadel reagent did not maintain their advantage over smaller dosages after a comparatively long time.

Bleaching with ultra-violet radiation.—The effect of sunlight in bleaching flour has long been recognized, but few data have been reported in the literature giving quantitative results after definitely timed exposures. Shutt (1911) placed flour one-quarter inch thick between glass plates and exposed it to sunlight. He stated that a bleaching was noticed at the end of one hour. He did not, however, report gasoline color values and he appears to have reached his conclusions merely by observing the visual appearance. As the effect of sunlight in bleaching flour is probably due chiefly to the ultra-violet rays, it

appeared of interest to measure the bleaching action of ultra-violet radiation.

The procedure used in these experiments was to expose a layer of flour, one-quarter inch thick or less, to the action of a source of ultra-violet radiation placed approximately 12 inches from the flour. At intervals of a half hour the samples were mixed and spread out again in a thin layer. Two light sources were used, a description of which follows.

One source of ultra-violet radiation was a Cooper Hewitt "Lab-Arc" lamp. The mercury arc is formed in fused quartz, the light source area being approximately ¼ x ¾ inches. This lamp is small compared to some others, but it emits the same characteristic radiation. It was operated on 110 volts, A.C., under which conditions it draws normally a current of one ampere. The burner begins as a low-pressure arc, but on heating it changes to a high pressure, in which case the ultra-violet radiation is intensified. In the experiments reported here, the flour was exposed after the lamp had been turned on for 15 minutes, when it was hot.

The second source of ultra-violet radiation was a Hanovia ultra-violet lamp of the so-called "Universal Laboratory model." This lamp was operated at 110 volts, D. C., under which it has a capacity of 5 amperes. This lamp is equipped with a polarity indicator, which enables one to note when the lamp is operated at its maximum efficiency. The burner is of the all-quartz type with ground-in Invar electrodes. It gives an arc of 6.5 cm.

The data secured in bleaching a sample of straight grade flour, No. 100, and Marquillo wheat flour are reported in Table XXIII. With the straight grade flour bleached under the Cooper Hewitt lamp, a 5-hour exposure reduced the carotin concentration 2.5 per cent. The same lamp used as a source of radiation in bleaching Marquillo wheat flour gave a bleach of 10 per cent at the end of 5 hours. The greatest bleaching effect was secured with the Hanovia lamp, which bleached 25 per cent of the original carotin concentration in Marquillo wheat flour after 4½ hours and 47 per cent after 7 hours. It is apparent that ultra-violet radiation is an effective bleaching agent, and decolorizes the carotin in flour.

It is not intended, in reporting these results on the influence of ultra-violet radiation as a bleaching agent, to compare the two light sources. The Cooper Hewitt lamp contained not only a smaller electrode than the Hanovia lamp, but it was 3 years old, while the Hanovia lamp was new. As the amount of ultra-violet radiation passed by the quartz

burner decreases with the age of a lamp and the extent to which it has been used, the Cooper Hewitt lamp probably was much less effective than a new lamp of this type would have been. It had been used continuously during the 3 years.

The intensity of the ultra-violet radiation emitted by the two sources was not definitely known, but the conditions under which they operated, as described above, were known. It would be desirable to calibrate the light source in making measurements of the type here reported.

TABLE XXIII

EFFECT OF BLEACHING FLOUR WITH ULTRA-VIOLET RADIATION AS SHOWN BY THE PER CENT TRANSMITTANCY OF THE GASOLINE EXTRACTS AND THE CONCENTRATION OF CAROTIN IN PARTS PER MILLION OF FLOUR

Determined in a 10-cm. cell at a wave length of 435.8 mμ.

	Straight grade flour, No. 100		Marquillo wheat flour Two samples			
	Cooper Hewitt "Lab-Arc" light source		Cooper Hewitt "Lab-Arc" light source		"Hanovia" light source Universal Lab. model	
	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million	Per cent transmittancy	Carotin, parts per million
Unbleached flour	16.0	2.08	1.8	4.56	4.1	3.64
After 4½ hrs. exposure	....	....	....	....	9.2	2.73
After 5 hrs. exposure	17.0	2.00	2.9	4.10	....	....
After 7 hrs. exposure	....	....	....	....	18.3	1.94
Carotin bleached in 4½ hours	.....		.....		25 per cent	
Carotin bleached in 5 hours	2.5 per cent		10 per cent		.....	
Carotin bleached in 7 hours	.. ...		.....		47 per cent	

The data on the bleaching effect of ultra-violet radiation on Marquillo wheat flour are plotted graphically in Figure 7. Time in hours is plotted along the axis of abscissa and concentration of carotin in parts per million along the axis of ordinate. The slope of the curve serves as an indication of the efficiency with which ultra-violet radiation bleaches the carotin of flour.

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